Factors Initiating Phytoplankton Blooms and Resulting Effects on Dissolved Oxygen in Duwamish River Estuary Seattle, Washington

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GEOLOGICAL SURVEY WATER-SUPPLY PAPER 1873-A

Prepared in cooperation with the Municipality of Metropolitan Seattle

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By EUGENE B. WELCH

ENVIRONMENTAL QUALITY

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CONTENTS

ILLUSTRATIONS

TABLES

ENVIRONMENTAL QUALITY

FACTORS INITIATING PHYTOPLANKTON BLOOMS AND RESULTING EFFECTS ON DISSOLVED OXYGEN IN DUWAMISH RIVER ESTUARY SEATTLE, WASHINGTON

By EUGENE B. WELCH

ABSTRACT

Phytoplankton productivity, standing stock, and related environmental factors were studied during 1964–66 in the Duwamish River estuary, at Seattle, Wash., to ascertain the factors that affect phytoplankton growth in the estuary; a knowledge of these factors in turn permits the detection and evaluation of the influence that effluent nutrients have on phytoplankton production. The factors that control the concentration of dissolved oxygen were also evaluated because of the importance of dissolved oxygen to the salmonid populations that migrate through the estuary.

Phytoplankton blooms, primarily of diatoms, occurred in the lower estuary during August 1965 and 1966. No bloom occurred during 1964, but the presence of oxygen-supersaturated surface water in August 1963 indicates that a bloom did occur then.

Nutrients probably were not the primary factor controlling the timing of phytoplankton blooms. Ammonia and phosphate concentrations increased significantly downstream from the Municipality of Metropolitan Seattle's Renton Treatment Plant outfall after the plant began operation in June 1965, and concentrations of nitrogen and phosphorus were relatively high before operation of the Renton Treatment Plant and during nonbloom periods. The consistent coincidence of blooms with minimum fresh-water discharge and tidal exchange during August throughout the study period indicates that bloom timing probably was controlled mostly by hydrographic factors that determine retention time and stability of the surface-water layer. This control was demonstrated in part by a highly significant correlation of gross productivity with retention time (as indicated by fresh-water discharge) and vertical stability (as indicated by the difference between mean surface and mean bottom temperatures). The failure of a bloom to develop in 1964 is related to a minimum fresh-water discharge that was much greater than normal during that summer. Hydrographic factors are apparently important because, as shown by studies of other estuarine environments by other workers, phytoplankton production increases when the zone of vertical turbulent mixing is not markedly deeper than the compensation depth.

Phytoplankton cells produced in the surface waters sink, thereby contributing oxidizable organic matter to the bottom saline-water wedge. The maximum BOD

(biochemical oxygen demand) in this bottom wedge occurs in the same section of the estuary and at the same time as the maximum phytoplankton biomass (as indicated by chlorophyll *a*) and minimum DO (dissolved oxygen). Other sources of BOD occur in the estuary, and conditions of minimum discharge and tidal exchange assist in reducing DO. Nonetheless, the highly significant correlation of chlorophyll *a* with BOD throughout the summer indicates that respiration and decomposition of phytoplankton cells is clearly an important contributor of BOD.

Increases in the biomass and resultant BOD of blooms because of increased effluent nutrients presumably would further decrease the concentration of DO. This possible effect of effluent nutrients was evaluated by laboratory bioassays and by a comparison of mean annual biomasses in the estuary. A green algal population in vitro did increase in response to added effluent nutrients; however, the available field data suggest that a 46-percent increase in effluent discharge between 1965 and 1966 did not increase the estuary's phytoplankton biomass significantly.

INTRODUCTION

PURPOSE AND SCOPE

A cooperative program between the Municipality of Metropolitan Seattle (Metro) and the U.S. Geological Survey was initiated in 1963 to study the variation in biological populations in the Duwamish River estuary in relation to water quality changes due particularly to domestic and industrial waste-disposal and treatment practices.

To determine the influence of physical factors on phytoplankton in the estuary, in situ productivity and standing stock measurements were studied in relation to the physical variables of water stability, retention time, and light transmittance. These variables were indicated by measurements of (1) salinity and temperature at different depths in a profile, (2) volume of fresh-water discharge, (3) tidal-prism thickness, (4) incident solar radiation, and (5) water transparency. To evaluate the effect of effluent nutrients on phytoplankton, the relation of productivity and standing stock measurements to phosphorus and nitrogen concentrations was studied in situ during periods of active phytoplankton growth. Further indications of the effect of the effluent were obtained from in vitro bioassays in which the quantity of added effluent was varied under controlled conditions. Phytoplankton standing stock and BOD (biochemical oxygen demand) distribution were related to the timing and location of DO (dissolved oxygen) minima to determine the importance of phytoplankton in causing oxygen depressions. This report evaluates possible influences of changes in water quality (especially increased nitrogen and phosphorus) on phytoplankton production and the resulting effect on dissolved-oxygen conditions in the estuary.

DESCRIPTION OF THE ESTUARY

The area studied includes the lower 21 km (kilometers) of the Duwamish River at Seattle, Wash. (fig. 1). This river section has a surface area of about 2.6 sq km (square kilometers) and ranges in depth from less than 1.0 m (meter) at the upper end to 17 m near the mouth; its mean depth is 9.5 m. The volume is estimated to be $11.2 \times 10^6 m^3$ (cubic meters). In the transition zone where the river enters the lower 10 km of the estuary—a reach that has been dredged for navigation—the velocity of flow decreases. Inorganic and organic particulate matter are deposited in this transition zone just upstream from the navigable reach and contribute to the depression of dissolved-oxygen concentrations. These conditions will be discussed in detail later in the report.

Five regular sampling stations in the river will be referred to by their distances—1.9, 7.7, 10.4, 12.6, and 21.0 km—above the mouth (fig. 1). The effluent from Metro's RTP (Renton Treatment Plant) enters the river 20.4 km above the mouth.

Fresh-water discharge ranged from 425 to 20,400 m^3/min (cubic meters per minute) during 1963–66. The mean tidal range recorded 6.5 km above the mouth was 2.3 m. The relatively large tidal range and discharge rate during most of the year result in conditions of great turbulent mixing and flushing, which upset the salinity and temperature patterns. The entire 21-km reach is affected by the tide, but the saline-water wedge intrudes only the lower two-thirds of the reach and then only at high tide and low discharge. Stoner (1967) has described conditions that permit saline-water intrusion to kilometer 12.6. Under these conditions saline water is always present at high tide when discharge is less than 1,060 m^3/min, but is never present when the discharge is greater than 1,700 m^3/min. Between these two discharge rates, saline intrusion depends on discharge and tide height. Water stability and retention time increase in the estuary as the period of low summer discharge is approached and during periods of low tidal exchange (high-low and low-high tides). Conditions of increased stability are indicated by increased differences in specific conductance and temperature with depth. During these low-discharge conditions, water dyed with rhodamine-B takes about 5 days to traverse the 21 km estuary.

BACKGROUND INFORMATION ON CONDITIONS IN THE ESTUARY

The Duwamish estuary receives waste effluents that affect its water quality. The upland flow into the estuary is a dilute soft water, but it carries some municipal, industrial, and agricultural byproducts. In ad-

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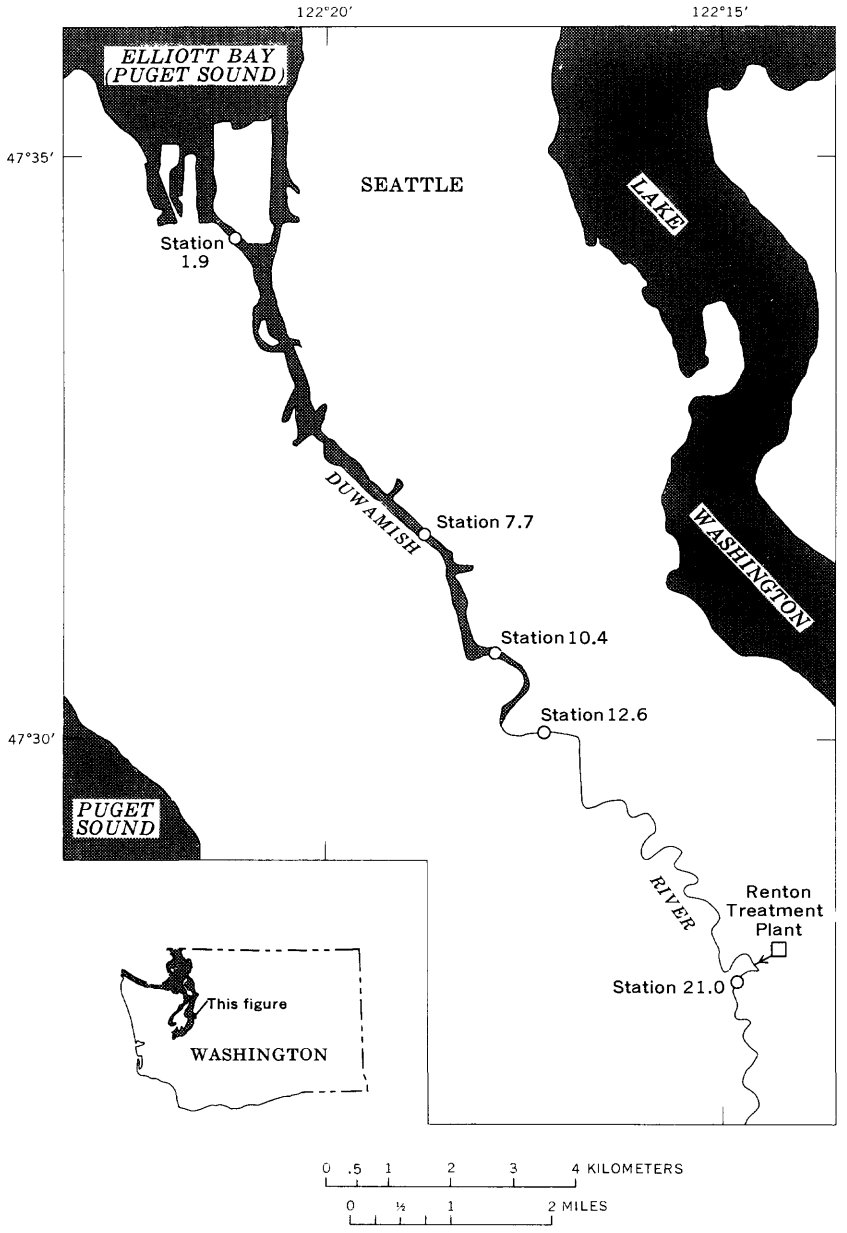

FIGURE 1.—Map of Duwamish River and vicinity, Washington, showing principal sampling stations (in kilometers above river mouth) and the Renton Treatment Plant.

dition, many sewers discharge untreated material into the lower estuary. In June 1965, Metro's RTP, which provides secondary treatment for sewage from a large area adjacent to Lake Washington, started discharging effluent into the Duwamish River. This plant is part of Metro's extensive system of waste-disposal and treatment facilities designed to eliminate the discharge of treated wastes into Lake Washington and of untreated wastes into Puget Sound.

Conditions of low DO have frequently occurred in the lower estuary during late summer (Okey, 1957; Isaac and others, 1964). There is concern that any further depression of DO during that period of the year will interfere with the migration of chinook and coho salmon. By about 1970, as the Metro waste-disposal system nears completion, all waste effluents presently discharging into the Duwamish below the RTP (mostly near the mouth) will be intercepted and discharged into Puget Sound following primary treatment. Though the treated effluent discharge from the RTP will continue to increase, the total BOD added to the estuary will ultimately be greatly reduced. In the meantime, the RTP effluent is superimposed on the other wastes in the estuary.

In 1966 the RTP operated at only about 9 percent of its ultimate capacity of 545,000 m^3/day (cubic meters per day), and the effluent discharge was about 8 percent of the ultimate quantity of the minimum river discharge, but the efficiency of BOD removal by the RTP was high. The average effluent BOD during 1966 was 10.5 mg/l (milligrams per liter), which represents only 8 percent of the influent BOD. The removal of mineral nutrients, however, was only 16 percent for ammonia and 3 percent for phosphate. The average effluent concentrations of ammonia and phosphate during 1966 were 15.5 mg N/l (milligrams of nitrogen per liter) and 9.5 mg P/l (milligrams of phosporus per liter), respectively. The average nitrate concentration in the effluent during 1966 was only 0.24 mg N/l (G. T. Mason, Metro, written commun., 1967).

Because BOD contribution by the RTP is small, the effluent was expected initially to have only a slight direct effect on dissolved-oxygen concentrations in the estuary. The relatively high nutrient content of the effluent, however, should result in increased nutrient concentrations downstream from the outfall. Therefore, if the effluent has any direct biological effect, there should be increased production of phytoplankton through utilization of the added nutrients. Nuisance blooms and eutrophication are well known problems that develop from increased phytoplankton production, frequently as a result of nutrients in domestic waste (Hasler, 1947; Edmondson and others, 1956; Bartsch, 1960; Barlow and others, 1963; Shapiro and Ribeiro, 1965; Oglesby and Edmondson, 1966; and Oswald and Golueke, 1966).

Physical factors such as stability, retention time, and transparency of the water mass are particularly critical to estuarine phytoplankton production. The timing of phytoplankton blooms in oceanic environments (particularly in the spring), as well as estuarine environments, is determined by the relations between depth of turbulent mixing, available light, and depth at which production equals respiration (termed compensation depth; Riley, 1942, Sverdrup, 1953, and Anderson and Banse, 1961). Gran and Braarud (1935) found that in waters which had a high tidal range and strong vertical mixing, phytoplankton were prevented from accumulating in the illuminated zone. These conditions were unfavorable for production even though nutrient concentrations were always high. Because the tidal range, flushing rate, and turbulent mixing are relatively great in the Duwamish even at low river discharge (Brown and Caldwell, 1958) and because nutrient concentrations were high before the effluent was introduced, a moderate increase in estuarine nutrient concentrations following activation of the RTP was not expected to alter bloom timing. The total phytoplankton biomass of a bloom might increase, however, as a result of a nutrient increase, thereby contribute to the BOD, and further depress the oxygen concentration in the estuary.

ACKNOWLEDGMENTS

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The assistance provided by project personnel of the U.S. Geological Survey and of Metro contributed greatly to this study. In particular, Mr. Gary W. Isaac of Metro offered many helpful suggestions and furnished assistance in the collection of the data. Critical review of the manuscript by Doctors Max Katz, Richard Van Cleve, and Alan C. DeLacy of the University of Washington and Dr. Keith V. Slack of the U.S. Geological Survey is most appreciated. In addition, Doctors W. T. Edmondson and Karl Banse of the University of Washington kindly reviewed results on various occasions during the study.

PROCEDURES AND METHODS

Procedures for the collection and analysis of water samples varied during the period of study. Because this was a cooperative project between Metro and the U.S. Geological Survey, both agencies contributed to the collection of data. The sample-collection schedule also changed as the project progressed and an increased understanding was gained of the variability and importance of some of the measurements. The data which specifically relate to the study of phytoplankton are included in table 1.

SAMPLING

Water samples for the determination of chlorophyll *a*, phytoplankton cell number, specific conductance, and temperature were collected on a reasonably consistent schedule, particularly after 1964 (table 1). During 1964, samples for the determination of these four variables were collected about twice a month, usually at a midtide stage. During 1965–66, samples for the determination of nutrients were collected monthly during the winter months and biweekly during the remaining time at both high and low tide. Samples were also collected from the saline-water wedge (1 m above the bottom during 1965–66 and 5 m below the surface in 1964) at stations 1.9 and 7.7 for determination of all variables except nutrients.

The sampling procedure for nutrients varied slightly from year to year (table 1). Metro personnel collected samples from the five principal stations biweekly during 1963–65 and monthly during 1966, but no collections were made between January and April in 1963 and 1964. Data from the Metro collections included determinations of nitrate, ammonia, and total phosphorus. During 1965–66, soluble phosphate and, occasionally, orthophosphate were determined. Metro samples were collected without regard to tidal stage, except for a few times in 1966. In 1965, samples were collected by the Geological Survey at high and low tide at the same stations where phytoplankton samples were collected; they were analyzed for nitrate and soluble phosphate. In 1966, ammonia and most of the other nutrient forms were determined in samples collected at high and low tide. Samples for phosphate determination were "fixed" with chloroform, and all samples were kept on ice or were refrigerated until analyzed.

The procedures for estimating primary productivity rates differed between years and between stations (table 1). (For a definition of the terms primary, net, and gross productivity, see Strickland, 1960, p. 6–8.) Samples for determination of productivity and chlorophyll *a* were collected at stations 1.9, 7.7, 12.6, and 21.0 bimonthly during the winter months, and monthly during the rest of the year. At the same time, Secchi-disk measurements were made at stations 1.9 and 7.7. In 1966, samples were collected at all stations for determination of total carbon dioxide. Samples were collected with a Van Dorn bottle at the surface and from depths of 1, 2, 3, and 4 m at the lower stations (1.9 and 7.7; fewer depths were sampled for productivity determinations from July to October 1964; see table 1) and at the surface and from a depth of only 1 m at the upper stations. Productivity profiles and light-attenuation estimates indicate that the depth range from the surface to about 4 m usually includes most of the photic zone. Measurements were limited to 1 m or less at the upper stations because the maximum

TABLE 1.—*Schedule for collection of water samples during 1963–66*

[Samples, unless specified, were collected without regard to tide stage]

Months	Variables measured	Stations sampled (km)	Depth (meters)	Frequency
		1963		
April to December	NH_3, NO_3, total PO_4	All five [1]	1	Biweekly.
Mid-August to October	Dissolved oxygen (DO)	7.7	1; 1 above bottom	Daily.
		1964		
April to December	NH_3, NO_3, total PO_4	All five [1]	1 [2]	Biweekly.
July to December	Chlorophyll *a*, temperature, cell count, specific conductance.	do	1 [2]	Semimonthly (at midtide stage during August-December).
July	Productivity, chlorophyll *a*	1.9; 7.7	0.6, 1.5, 3.0	Monthly.
August	do	1.9; 7.7	0.6, 1.2, 2.4, 3.0	Do.
Do	do	12.6; 21.0	0.6	Do.
October to December	do	1.9; 7.7	0,[3] 1, 2, 3, 4	Bimonthly.
Do	do	12.6; 21.0	0,[3] 1	Do.
July to mid-August	Daily minimum DO	7.7	Less than 1 above bottom.	Weekly.
Mid-August to September	do	7.7	0.9 above bottom	Daily (automatic monitor).
		1965		
All	NH_3, NO_3, total PO_4	All five [1]	1 [2]	Biweekly.

January to February; September to December.	Chlorophyll *a*, cell count, temperature, specific conductance, NO_3, soluble PO^4.	---do---	1 [2]	Monthly at high and low tide.
March to August	do	do	1 [2]	Biweekly at high and low tide.
January to February; November to December.	Productivity, chlorophyll *a*, Secchi disk.	1.9; 7.7	0, 1, 2, 3, 4	Bimonthly.
Do	do	12.6; 21.0	0, 1	Do.
March to October	do	1.9; 7.7	0, 1, 2, 3, 4	Monthly.
Do	do	12.6; 21.0	0, 1	Do.
July to September	Daily minimum DO	7.7	Less than 1 above bottom.	About three per month.
	1966			
January to April; October to December.	NH_3, NO_3, total PO_4, soluble PO_4.	All five [1]	1 [2]	Monthly.
Do	do	do	1 [2]	Monthly at high and low tide.
May to September	do	do	1 [2]	Biweekly at high and low tide.
January to April; September to October.	Chlorophyll *a*, cell count, temperature, specific conductance.	do	1 [2]	Monthly at high and low tide.
May to August	do	do	1 [2]	Biweekly at high and low tide.
February to September	Productivity, chlorophyll *a*, Secchi disk, total CO_2.	1.9	0, 1, 2, 3, 4	Monthly.
Do	Productivity, chlorophyll *a*, total CO_2.	12.6; 21.0	0, 1	Do.

Footnotes a t end of table.

TABLE 1.—*Schedule for collection of water samples during 1963–66*—Continued

[Samples, unless specified, were collected without regard to tide stage]

Months	Variables measured	Stations sampled (km)	Depth (meters)	Frequency
		1966—Continued		
February to June; September.	Productivity, chlorophyll *a*, total CO_2.	12.6; 21.0	0, 1	Monthly.
Do	Productivity, chlorophyll *a*, Secchi disk, total CO_2.	7.7	0, 1, 2, 3, 4	Do.
July to August	do	7.7	0, 1, 2, 3, 4	Biweekly.
June to September	Chlorophyll *a*, BOD, DO	Eight [4]	Less than 1 above bottom.	Weekly at high and low tide.
Do	do	1.9; 7.7	0	Do.
Do	Temperature, specific-conductance profiles.	7.7	Intervals of 0.9	Three per month.
June to August	BOD, DO	7.7	1; 1 above bottom	Biweekly at high and low tide.
June to September	Daily minimum DO	7.7	0.9 above bottom	Daily (automatic monitor).

[1] Stations 21.0, 12.6, 10.4, 7.7, and 1.9 (kilometers above the mouth of the Duwamish River).

[2] Samples were collected 1 m above stream bottom (5 m below surface in 1964) at stations 1.9 and 7.7 for determination of chlorophyll *a*, cell count, temperature, and specific conductance at all times when samples for these determinations were collected 1 m below the surface.

[3] 0 meter depth omitted in October samples.

[4] Stations 10.4, 10.2, 10.0, 9.7, 7.7, 6.4, 4.3, and 1.9.

depth of the river was only about 1 m during low flow. The light- and dark-bottle oxygen method was used to estimate productivity at all stations in 1964 and 1965, but only at station 7.7 in 1966. Estimates of productivity by the carbon-14 method were made at all stations in 1966. Duplicate light-bottle samples were collected at each depth for use with both methods, but duplicate dark bottles were used only with the oxygen method. Samples were incubated in situ for 24 hours (48 hours in the winter) for use with the oxygen method and 4 hours for use with the carbon-14 method). Incubation times using the oxygen method were shorter (6–8 hrs) in July and August 1964. Incubation depths (with the exception of July–October 1964) were 0.25, 1, 2, 3, and 4 m at the lower two stations and 0.25 and 1 m at the upper three stations.

The incubating bottles were attached to a weighted line which hung vertically from the center of an aluminum bar suspended between polyurethane floats. This unit was attached to the bridge structure at each station in a manner that permitted free vertical movement with the tide. The arrangement maintained the bottles satisfactorily under conditions of fluctuating tidal stage and current.

Data were collected during June–September 1966 to study the relation between phytoplankton standing stock (as indicated by chlorophyll *a* concentration), DO, and BOD (table 1). Water samples were collected, usually weekly, at high and low tide less than a meter above stream bottom at stations 1.9, 4.3, 6.4, 7.7, 10.0, 10.2, and at 10.4 km above the mouth. At the same time, surface samples were collected at stations 1.9 and 7.7. In addition, DO, BOD, chlorophyll *a*, nutrients, temperature, and specific conductance were determined in samples collected biweekly from the surface and bottom at station 7.7.

The "potential" of water to produce phytoplankton was studied under controlled conditions. Duplicate surface or 1-m water samples were collected at station 7.7 during August 1966 along with scheduled chlorophyll samples. The additional samples were incubated in vitro under conditions of uniform light (about 7,000 lux) and temperature (20°–21°C), without mixing for 5 or 6 days. After this period, chlorophyll *a* concentration was determined and compared with the initial concentration.

Surface-water samples were collected from stations 7.7, 12.6, and 21.0 on March 1 and June 3, 1966, for a study of the effect that RTP effluent had on phytoplankton production in vitro. Samples were filtered through HA Millipore filters of an average 0.45μ (micron) porosity. Sufficient filtered effluent was added to three 500-ml (milliliter) flasks containing water from station 7.7 to obtain concentrations of 5, 10, and 25 percent effluent in a final volume of 400 ml. No effluent

was added to three other flasks containing filtered water from stations 7.7, 12.6, and 21.0. Each of these flasks was inoculated with 5 ml of river water from station 7.7. Sodium chloride was added in various amounts to filtered water in one sample series to equalize salinity between the flasks. Radioactive carbon in sodium carbonate form was added to each flask at a concentration of 50μc/l (microcuries per liter) to measure carbon assimilation according to the procedure by Goldman and Carter (1965, p. 1056). The flasks were incubated for about 8 days under conditions of uniform light (about 7,000 lux) and temperature (20°–21°C) with moderate mixing. Aliquots of 25 or 50 ml were removed from each flask daily. These were then filtered and the activity of the filters was determined as a relative measure of carbon assimilation.

Profiles of specific conductance and temperature were determined in situ at station 7.7 three times per month at high and low tide, from June to September 1966 (table 1). These data were used as an indicator of vertical stability.

Automatic water-quality monitors have operated periodically at stations 1.9, 7.7, 12.6, and 21.0 from August 1964 through 1966. Water for analysis is pumped into the monitors from 0.9 m below the surface at all stations and from 0.9 m above the bottom at the lower two stations (1.9 and 7.7). The monitor determinations of DO (table 1), temperature, and specific conductance were used to supplement data obtained biweekly or monthly by manual sampling and to study daily changes in the variables at station 7.7. Solar radiation measured with an Eppley pyrheliometer was recorded continuously by the monitor at station 12.6.

ANALYSIS AND COMPUTATION

Nutrient concentrations were determined by Metro according to methods defined by the American Public Health Association and others (1965). To show seasonal variability in nutrient concentrations, data collected by different sampling procedures were grouped to facilitate comparison. Where determinations are available for samples collected at high and low tide, a mean of the two values is included with data that are collected without regard to stage of tide. To illustrate variability due to tide, season, and conditions of river flow, both the high- and low-tide values are given.

Mean concentrations of ammonia and total phosphate were compared between stations and between years both by graph and by analysis of variance to determine if a significant increase occurred in posteffluent nutrient concentrations. For this purpose, ammonia and

total phosphate data from the preeffluent period, January–April 1965, were used for the same months in 1963 and 1964 for which data were not available. The use of these data permitted comparisons between annual mean concentrations before and after introduction of the effluent. Nutrient content is low and relatively constant during January–April because fresh-water discharge is high. Thus, actual nutrient values during that period in 1963 and 1964 probably differed little from those in 1965.

The Alsterberg modification of the Winkler method (American Public Health Association and others, 1965) was used to determine DO in samples collected with the Van Dorn bottle. The concentrations (with corrections for salinity and temperature) recorded by the monitors were determined automatically by a polarographic oxygen electrode.

BOD was determined by the standard method (American Public Health Association, 1965). Duplicate samples were drawn from a Van Dorn bottle; one was analyzed for initial dissolved oxygen and the other incubated at 20°C for 5–6 days. In August 1966, when DO declined, duplicate samples were drawn from a Van Dorn bottle as usual, but one was saturated with oxygen (mechanically aerated) and the other was left unsaturated.

Chlorophyll *a* was determined by the method of Richards and Thompson (1952) as modified by Creitz and Richards (1955). The specific absorption coefficients reported by Parsons and Strickland (1963) were used to calculate chlorophyll *a* concentration. To show seasonal variation in chlorophyll *a*, the most important factor in the occurrence of blooms, the chlorophyll *a* data collected by slightly different sampling procedures were grouped to furnish a more complete record for comparison between the 3 years. The majority of the data are means of values determined from samples collected biweekly at both high and low tide. Also included are means of values determined from surface and 1-m samples collected without regard to tide (from productivity studies), and values determined from samples collected on a midtide stage (mostly in 1964).

The procedures of Strickland and Parsons (1960, p. 153) were followed in productivity determinations. When the oxygen method was used, estimates were expressed as gross productivity of equivalent carbon and were based on a photosynthetic quotient, $\triangle O_2/\triangle CO_2$, of 1.2. Daily productivity per square meter was estimated to a depth of 4 m at the two lower stations and to a depth of 1 m at the upper stations. A mean of each pair of productivity measurements at successive depths was assumed to represent the mean of the cubic meter between them; for example, a mean of the surface and 1-m values represents the first meter in the column. If fewer depths were sampled or if the incubation

periods were shorter than usual (July–October 1964), data were extrapolated to permit productivity estimates to a 4-m depth at the lower two stations and to a 1-m depth at the upper stations.

Anomalies in the productivity results based on the light- and dark-bottle oxygen method occurred particularly during winter, when photosynthetic activity was low. These anomalous results were modified slightly in computing a productivity estimate per square meter. Negative values for gross productivity (that is, dark-bottle oxygen concentration exceeding that of the light bottle) were treated as zero; however, where a negative respiration value (dark-bottle oxygen concentration exceeding the initial concentration) at a particular depth in the profile was associated with positive values at other depths in the profile, the negative value was replaced by the mean of the positive values. This was done because there is no logical explanation for negative respiration (an increase in oxygen content in the dark bottle). Assuming that organisms were fairly evenly distributed in the vertical profile when negative respiration values occurred, the respiration values should have been nearly equal at most depths. Under such conditions, a mean of the positive respiration values represents the best estimate of true respiration.

Standardized activity of the carbon-14 stock solution was determined by Dr. C. R. Goldman, professor of zoology at the University of California at Davis; he (1961, p. 104) used his total-filter combustion method. A Picker scaler equipped with a thin-window gas-flow β detector was used to count filter activity. Other variations in the methods of Strickland and Parsons (1960, p. 153) included the use of smaller bottles (125 ml) and smaller filters (25 mm). The estimates of carbon-14 productivity determined for the 4-hour incubations were extrapolated to daily productivity by using the ratio of total daily incident light to light during the incubation period.

Total inorganic carbon concentration, a figure necessary to compute productivity rates by the carbon-14 method, was determined gravimetrically with a carbon dioxide train (Goldman, 1961, p. 106). The error between duplicate samples with this method was usually less than ± 5 percent.

Phytoplankton cell concentrations in water samples preserved with Lugol's solution were counted. The counts were made by the sedimentation technique, using an inverted microscope (Lund and others, 1958). Cells greater than 5μ in the longest dimension were included in the counts. Usually, at least 50 cells each of diatoms and green algae were counted per sample. Limited data indicate that the error between duplicate observations is +10–20 percent and that most of this error is involved with subsampling the original sample. Diatom frustules

were included unless they were noticeably damaged. This procedure facilitated the distinction between "live" and "dead" cells. Diatoms and some green algae were usually classified to the generic level, but no attempt was made to classify spherical and flagellated green species. Computations were made for means of high- and low-tide concentrations, except for the 1964 data, when samples were collected on a mid-tide stage.

The mean daily solar radiation for each weekly period was computed from continuous data recorded by the monitor at station 12.6 and from supplementary data supplied by the Weather Bureau at the Seattle–Tacoma Airport. Light attenuation with depth in the water column was measured at station 7.7 with a submarine photometer and was compared with Secchi-disk measurements. This comparison provided a constant of 0.73 with which attenuation coefficients (K) could be computed from Secchi-disk measurements (D) according to the formula $K=0.73/D$. These data were used to compute the depth of the photic zone (the depth at which light intensity is 1 percent of the surface intensity) and the quantity of light reaching a particular depth.

The daily tidal-prism thickness was approximated by computing the difference between the sum of the two predicted high tides and the algebraic sum of the two low tides for each day. Fresh-water discharge was recorded continuously at station 21.0.

In the regular biweekly sampling, temperature was measured in the field with a pocket thermometer, and specific conductance (in micromhos per centimeter at 25°C) was measured in the laboratory with a conductivity bridge. Profiles of temperature and specific conductance were also measured in situ with a salinometer at depth intervals of 0.9 m.

Analysis-of-variance tests and correlation analyses were computed according to BMDO5V and BMDO2R digital computer programs that were prepared by the Health Sciences Computing Facility, University of California at Los Angeles.

EVALUATION OF METHODS

A comparison of BOD results from aerated and unaerated water samples indicated that the BOD was partly dependent on the initial DO. The mean BOD concentration was 1.5 mg/l in unaerated samples with an initial DO less than 4.0 mg/l and with a postincubation concentration greater than 0.0 mg/l. The mean BOD concentration in duplicate water samples that were aerated before incubation was 2.6 mg/l, or 75 percent greater. Zobell (1940) found that oxidation of organic matter by marine bacteria was not limited by oxygen concen-

trations between 0.3 and 12.7 mg/l. He suggested, however, that a low oxygen concentration could limit oxidation reactions in localized microspheres that are rich in oxidizable matter, such as decomposing plankton. This situation could occur if oxygen is consumed more rapidly than it is replaced by diffusion from surrounding water. Such a phenomenon may account for the apparent dependence of BOD on oxygen concentration since phytoplankton density was great during the period of low dissolved oxygen in the estuary.

Part of the variability in chlorophyll *a* measurements was due to the method of sample handling and analysis, as well as to diurnal variation in tidal stage. The variability due to the method was evaluated by analyzing 13 subsamples from a single river sample collected at station 7.7. The mean and standard errors of this series were 2.6 and 0.08 mg/m^3 (milligrams per cubic meter), respectively. Chlorophyll *a* was found to vary inversely with tidal stage in the middle part of the estuary, where the greatest diurnal changes in salinity occur. This relationship and possible causes are discussed in a paper by Welch and Isaac (1967).

Because of interference by detrital chlorophyll, the use of cholorophyll *a* concentration as an estimate of phytoplankton standing stock or metabolic density is sometimes questioned (Saunders and others, 1962, p. 47), but chlorophyll *a* concentration is considered a reliable estimate of the standing stock in the Duwamish because of the highly significant correlations between chlorophyll *a* and productivity. Regression analyses of productivity (carbon-14) data and of chlorophyll *a* data from all stations during 1966 (fig. 2) and of similar data (oxygen method) from all stations during 1964–65 (Welch and Isaac, 1967) show correlation coefficients of 0.97 and 0.96, respectively. The regression line intercept for both groups of data is near the origin; this relation indicates that very little detrital cholorophyll is present. The larger concentrations and higher productivity rates of chlorophyll *a* probably explain these highly significant correlations. Under these conditions, populations are actively growing, and in an estuary characterized by considerable flushing, such as the Duwamish, there is little time for old, inactive cells (detrital chlorophyll) to accumulate.

Great differences have been reported between productivity rates measured by the carbon-14 method and those measured by the oxygen method. Estimates of productivity by the carbon-14 method give values that usually are greater than net but less than gross productivity, according to Steemann Nielson and Hansen (1959). Experimental work by Thomas (1964) showed that the relation of carbon-14 to net and gross oxygen productivity depends on the growth stage and the supply of nutrients available to the population. Antia and others,

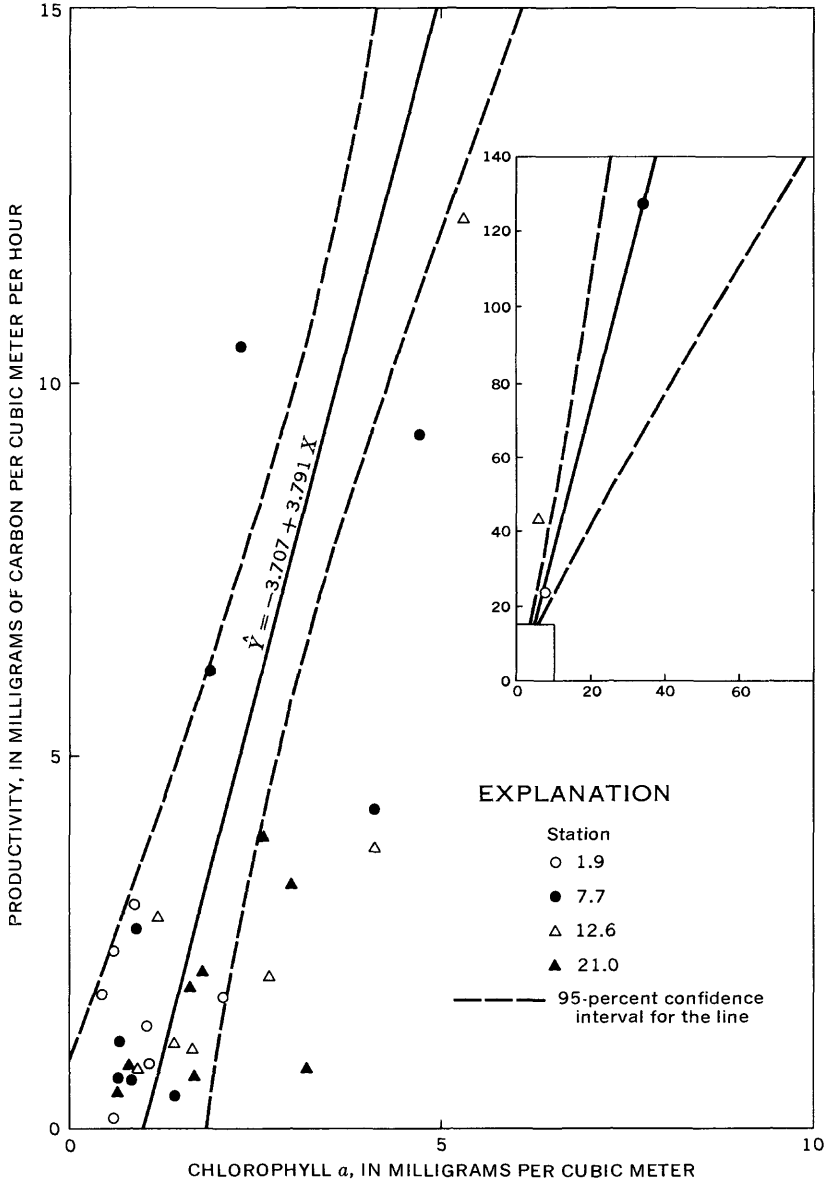

FIGURE 2.—Relation of phytoplankton-productivity rate to chlorophyll *a* concentrations at four stations during 1966. Productivity is measured by the carbon-14 method. Points are means of measurements taken at stream surface and 1 m below stream surface. Inset shows off-scale values. $\hat{Y}$ is the productivity and X is the chlorophyll *a* concentration.

(1963) found a great difference between oxygen evolution (as equivalent carbon) and carbon assimilation in a growing marine phytoplankton population isolated in a plastic sphere. The lower rate of carbon assimilation was attributed to excretion of dissolved organic matter (35 percent of the total fixed). A considerable difference between the two methods of estimating productivity rates was observed in the Duwamish during 1966, particularly during the bloom at station 7.7. The gross and the net productivity determined by the oxygen method were 4,700 and 3,700 mg C/m^2/day (milligrams carbon per square meter per day), respectively, while coincident productivity that was determined by the carbon-14 method was 1,500 mg C/m^2/day. At least part of the measured difference between the methods is probably due to nutrient excess and cellular excretion, which respectively result in increased oxygen evolution and a loss of fixed carbon from the cells.

RESULTS

PHYTOPLANKTON DISTRIBUTION

During much of the year, the phytoplankton standing stock is small in the Duwamish estuary and consists primarily of fresh-water species, for example, green algae, euglenophytes, desmids, and fresh-water pennate diatoms. During the late summer, however, the salinity is higher and conditions are satisfactory for dense nannoplankton blooms, primarily of marine diatoms. Figure 3 shows the distribution of these blooms, as indicated by chlorophyll *a*, both before and after the introduction of RTP effluent in June 1965. The standing stock was greatest at station 7.7. A maximum chlorophyll *a* concentration of 28 mg/m^3 was determined on a surface sample collected at station 7.7 at a midtide stage on August 18, 1965, whereas a maximum concentration of 70 mg/m^3 was determined at that station on August 16, 1966, at low tide. The greater chlorophyll *a* concentrations in 1966 may be due in part to more frequent sampling during the bloom period; this probably increased the chance of observing maximum densities.

A bloom did not occur in 1964, prior to the introduction of the RTP effluent. The maximum chlorophyll *a* concentration was only about 3.0 mg/m^3 at station 7.7 during that year. Although phytoplankton samples were not taken during 1963, a bloom probably did occur in August because DO at station 7.7 reached 92 and 152 percent of saturation in the afternoons on August 8–9 and 19–20, respectively. Single samples from 4 other days of the month showed percent-saturation maxima ranging from 88 to 109. (The daily maxima in September 1963 ranged from 60 to 74 percent.) Maximum daily oxygen saturation 0.9 m below the water surface at station 7.7 during the bloom of August 1966 were greater than 100 percent (figs. 3, 4); the maxima

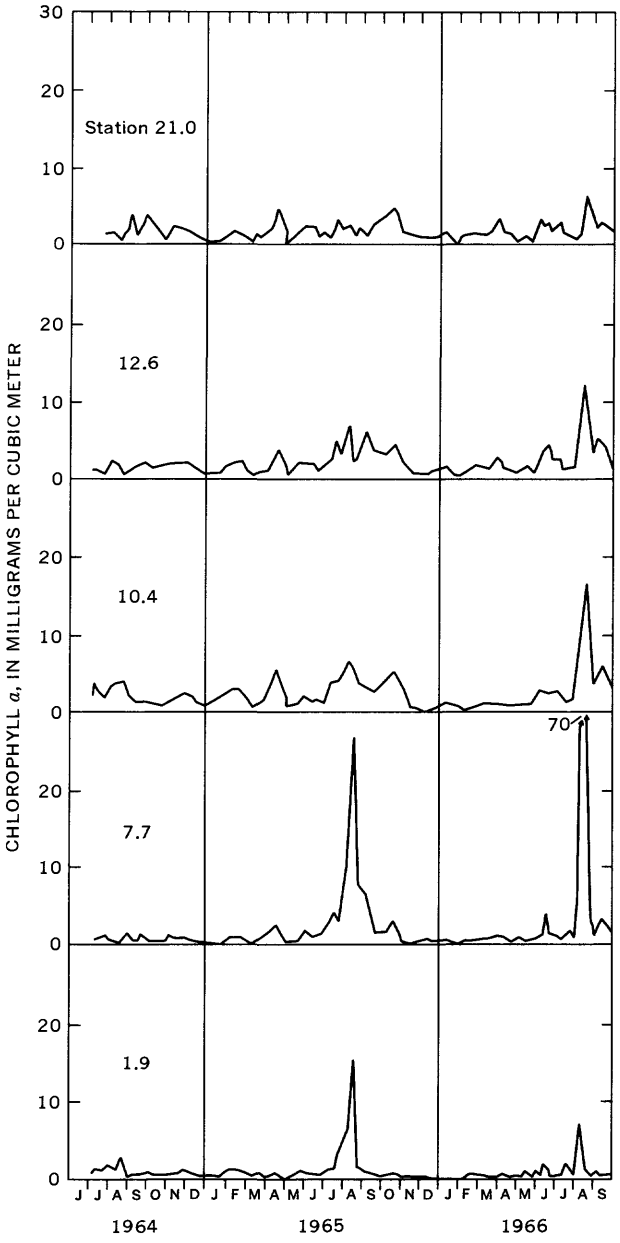

FIGURE 3.—Distribution of phytoplankton blooms during June 1964–September 1966, as indicated by the chlorophyll *a* concentrations at five stations.

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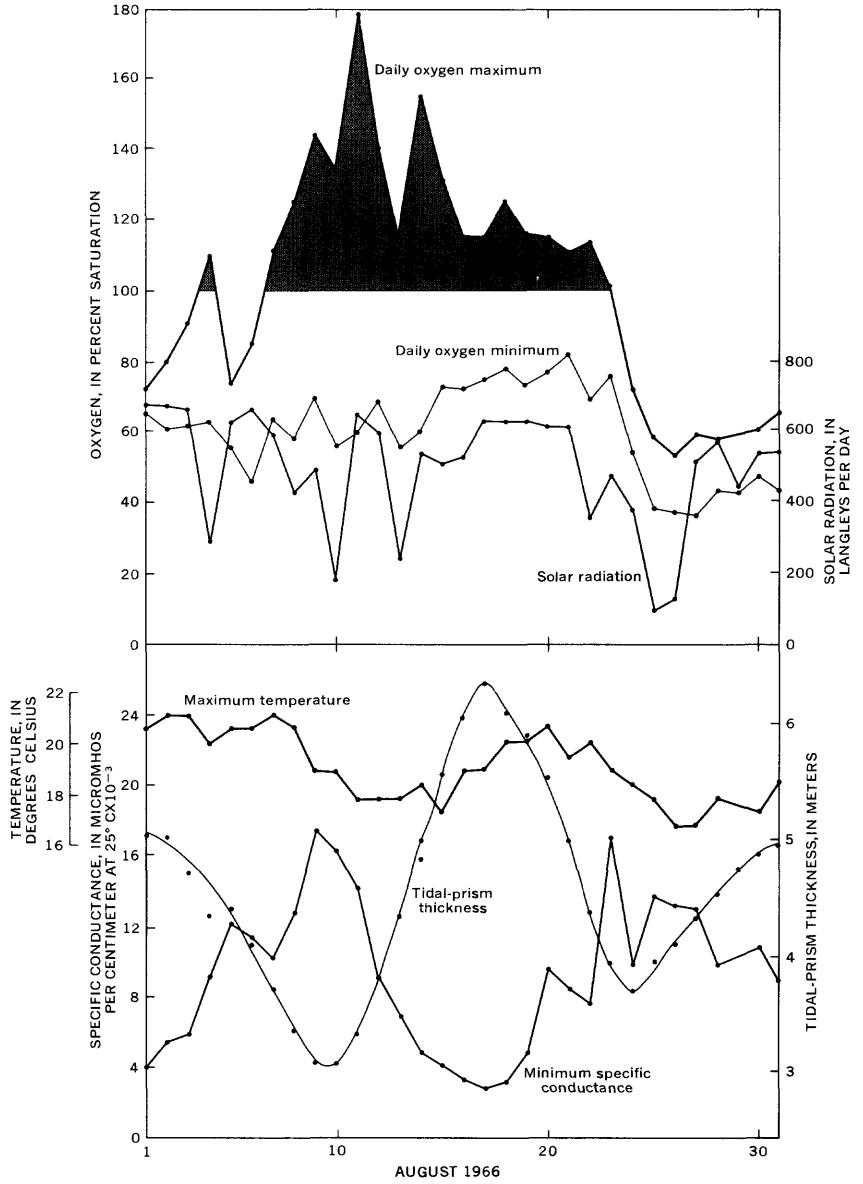


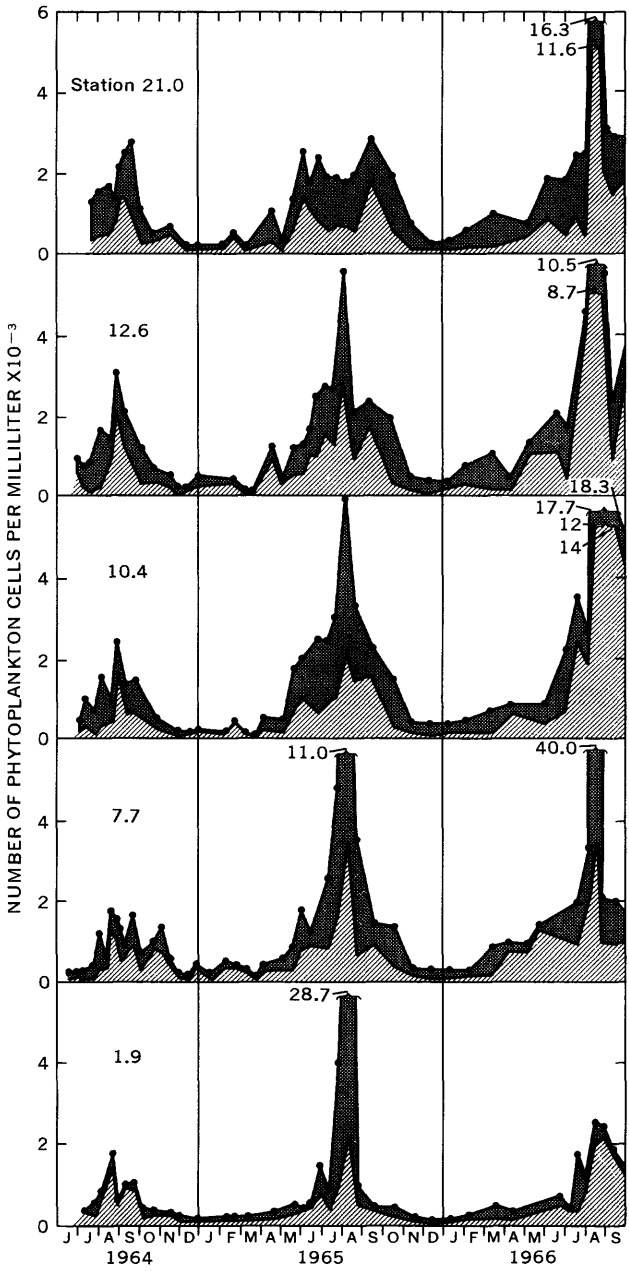
FIGURE 4.—Relation of phytoplankton activity to solar radiation, maximum water temperature, minimum specific conductance, and tidal-prism thickness at station 7.7 during the phytoplankton bloom in August 1966. Phytoplankton activity is indicated by percent saturation of oxygen.

during 1963 ranged from 60 to 152, therefore the saturation percentages in 1963 probably indicates a bloom during that year.

The distribution of phytoplankton during 1964–66 is further portrayed by cell counts (fig. 5). Diatoms were the most abundant organisms in the estuary, particularly during bloom periods. At station 7.7 on August 10, 1965, the most common diatom was *Skeletonema costatum*, which formed 82 percent of the 13,000 cells per milliliter at high tide. At the same station at low tide, unicellular green algae were more abundant; they formed 52 percent of the 9,000 cells per milliliter. At station 1.9 on this date, *Skeletonema* formed 92 and 93 percent of the 32,300 and 25,200 cells per milliliter at low and high tide, respectively. On August 24, however, neither *Skeletonema* cells nor total cells, were abundant at high or low tide at station 1.9 or at high tide at station 7.7. The low-tide sample at station 7.7 contained a greater number of cells than the high-tide samples, and a centric diatom (probably *Thalassiosira* sp.) constituted 63 percent of the total 5,500 cells per milliliter. Samples for cell counts were not taken on August 18, when maximum chlorophyll *a* concentrations were determined at station 7.7; however, total cell numbers were probably greater on August 18 than on August 10 or 24, and the two diatom species certainly must have represented a large part of the biomass.

In 1966, *Skeletonema costatum* was scarce; however, *Thalassiosira* was again abundant at station 7.7. At low tide on August 16, when the annual maximum chlorophyll *a* concentration (70 mg/m^3) was determined, *Thalassiosira* comprised 48 percent, while a green algal species, *Micratinium* sp., comprised 26 percent of the total 59,300 cells per milliliter. The total number of cells was less at high tide than low tide (20,600 cells per milliliter), but *Thalassiosira* still comprised 38 percent of the total. Unidentified unicellular green algae and flagellates comprised 59 percent of the total high-tide concentration, but *Micratinium* was scarce. Cell numbers at station 1.9 were smaller during the 1966 bloom period than during the same period in 1965. At low tide on August 16, 1966, *Thalassiosira* comprised 22 percent of the total 4,200 cells per milliliter, and species of green algae (some *Micratinium*) comprised 75 percent. The total number at high tide was only 900 cells per milliliter.

The centric diatom collected at stations 7.7 and 1.9 in 1965 was tentatively identified as *Thalassiosira* sp. because it was apparently limited in distribution to areas of higher salinity in the lower estuary and because it has the superficial characteristics of a member of that genus that is abundant in Puget Sound. In August 1966, however, a small centric diatom was observed in small numbers at the farthest upstream station (21.0), which is beyond the limit of salinity intrusion


Station 21.0
16.3
11.6
12.6
10.5
8.7
10.4
18.3
17.7
12
14
7.7
11.0
40.0
1.9
28.7
NUMBER OF PHYTOPLANKTON CELLS PER MILLILITER X10⁻³
0
2
4
6
J J A S O N D J F M A M J J A S O N D J F M A M J J A S
1964
1965
1966

but still is subject to variation in stage by tidal action. The presence of this diatom so far upstream suggests that the diatom may be of fresh-water origin, perhaps a species of *Stephanodiscus*. [Regardless, the organism flourishes in the brackish water of the lower estuary, and it has a wider salinity tolerance than would be expected for either of the two species.]

Productivity rates, like chlorophyll *a* concentrations, were greatest at station 7.7 during August 1965 and 1966 (fig. 6). In fact, productivity rates, chlorophyll *a* concentrations, and cell counts all show reasonable agreement in describing phytoplankton distributions in the estuary; however, cell counts during August and September 1966 were much higher at the upper stations (especially 21.0) than the chlorophyll *a* content might indicate (fig. 5). Most of the peak cell population at these stations consisted of very small unicellular green organisms. Figures 3 and 6 show that these high counts of green algae contributed proportionately much less chlorophyll *a* and productivity at the upper stations than the dominantly diatom population contributed at station 7.7.

Although the productivity rates shown in figure 6 are computed to a depth of 4 m at the lower two stations (1.9 and 7.7) but to only 1 m at the upper two stations, the rates are nonetheless comparable because the greatest productive area is in the first meter below the surface. During the bloom period in 1966, productivity estimates in the top cubic meter at stations 12.6 and 7.7 were 43 and 127 mg C/m^3/hr (milligrams carbon per cubic meter per hour), respectively. These rates are of the same magnitude relative to each other as are the rates shown for the stations 1.9 and 7.7 in figure 6. Yet, in that figure, the rate for station 7.7 represents a total production to a depth of 4 m, whereas the rate for station 12.6 applies to the production of only the top meter. Thus, regardless of sampling method, the greatest biomass and rate of productivity clearly occur at station 7.7.

Dense phytoplankton blooms occurred in the lower estuary downstream from the RTP outfall during both years following effluent introduction. To determine if the RTP effluent was instrumental in causing these blooms, studies were made of the physical and chemical factors that are known to affect phytoplankton production.

◀ FIGURE 5.—Distribution of phytoplankton, as indicated by cell concentrations at five stations, June 1964–September 1966. Water samples were collected 1 m below stream surface. Curves are based on means of samples taken at high and low tide during 1965–66, and single samples taken between high and low tide during 1964. Green algae and diatoms are indicated by crosshatching and shading, respectively.

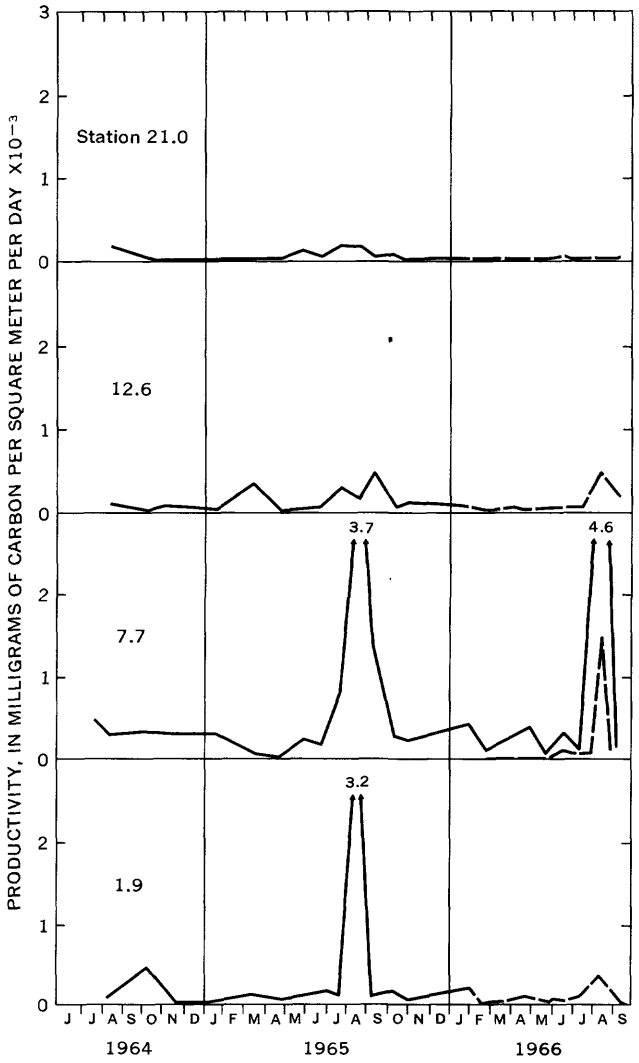


FIGURE 6.—Phytoplankton productivity at four stations, July 1964–September 1966. Productivity measured by the oxygen method gives gross values (solid line) and by the carbon-14 method gives values between gross and net (dashed line). Integrated water depths are: 4 m at stations 1.9 and 7.7 and 1 m at stations 12.6 and 21.0.

EFFECTS OF EFFLUENT NUTRIENTS

Phosphate, ammonia, and nitrate concentrations in the estuary before and after the introduction of effluent have been compared. Nutrient levels based on total weight for the various years cannot be

computed and compared because accurate water-discharge measurements are not available from the lower stations in the estuary; therefore, the nutrient levels are compared by graphical and statistical methods. The possible effects of the addition of effluent nutrients on phytoplankton production are evaluated by comparing in situ nutrient and phytoplankton observations in the Duwamish River estuary during 1964–66 and by experimenting with in vitro nutrient bioassays at the RTP laboratory during 1966.

Although silicate is important for the growth of diatoms, data on the relationship between the two in the Duwamish River are not included in this paper. The RTP did not contribute silicate to the river, but during the summer of 1966 concentrations of silicate remained high, ranging between 4 and 9 mg Si/l (milligrams silicon per liter) in the surface water at station 7.7.

PHOSPHORUS

Total-phosphate concentrations were greater downstream from the RTP outfall during 1965–66 than they were upstream. No such increase was obesrved at these midestuary stations during 1964, before effluent had been introduced (fig. 7). Some of the difference in phosphate concentrations between 1964 and 1965–66 can probably be related to fresh-water discharge, which was unusually high during the summer of 1964. The highest concentrations of total phosphate were measured about July 1, 1965, and about August 1, 1966, and both coincided with decreased fresh-water discharge. Because the inverse relationship of higher phosphate to fresh-water discharge was also observed above the RTP outfall at station 21.0, the differences in total phosphate between 1964 and 1965–66 must have been exaggerated by differences in discharge. However, not all phosphate differences before and after the introduction of effluent were due to discharge. A comparison of data during 1963 and 1965, when discharge conditions were similar, shows that the mean annual concentrations of total phosphate at stations 1.9–12.6 during 1963 were less than the comparable values during 1965. Station 21.0, which is above the RTP outfall, was the only exception (fig. 8). Also, figure 7 shows consistently higher total phosphate below the outfall during the winter of 1965–66 than during the winter of 1964–65.

Downstream changes in the concentrations of total phosphate before and after introduction of the RTP effluent have been compared by analysis of variance (table 2). If stations and periods are considered fixed, the increase in total phosphate in 1965–66 (after effluent introduction) and the differences in concentration between stations are significant at the 0.5-percent level. Interaction between periods and stations, however, is also significant at the 0.5-percent level. Part of the

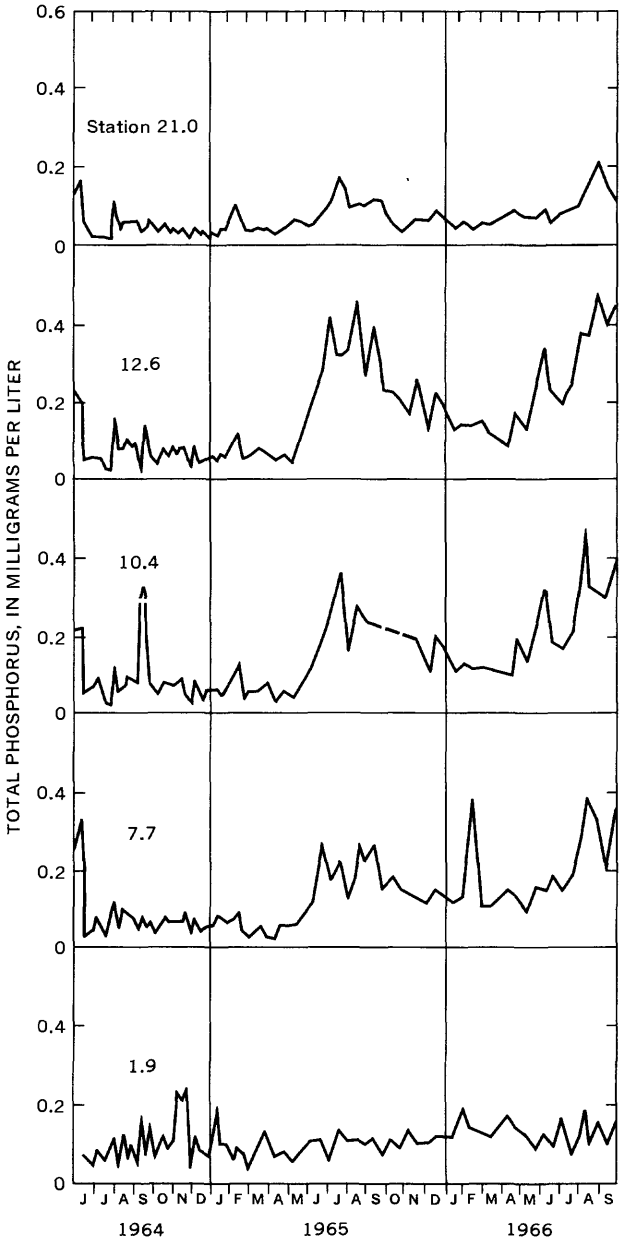


FIGURE 7.—Nutrient content of total phosphate, expressed as phosphorus, at five stations, June 1964–September 1966. Water samples were collected 1 m below stream surface, usually without regard to tidal stage.

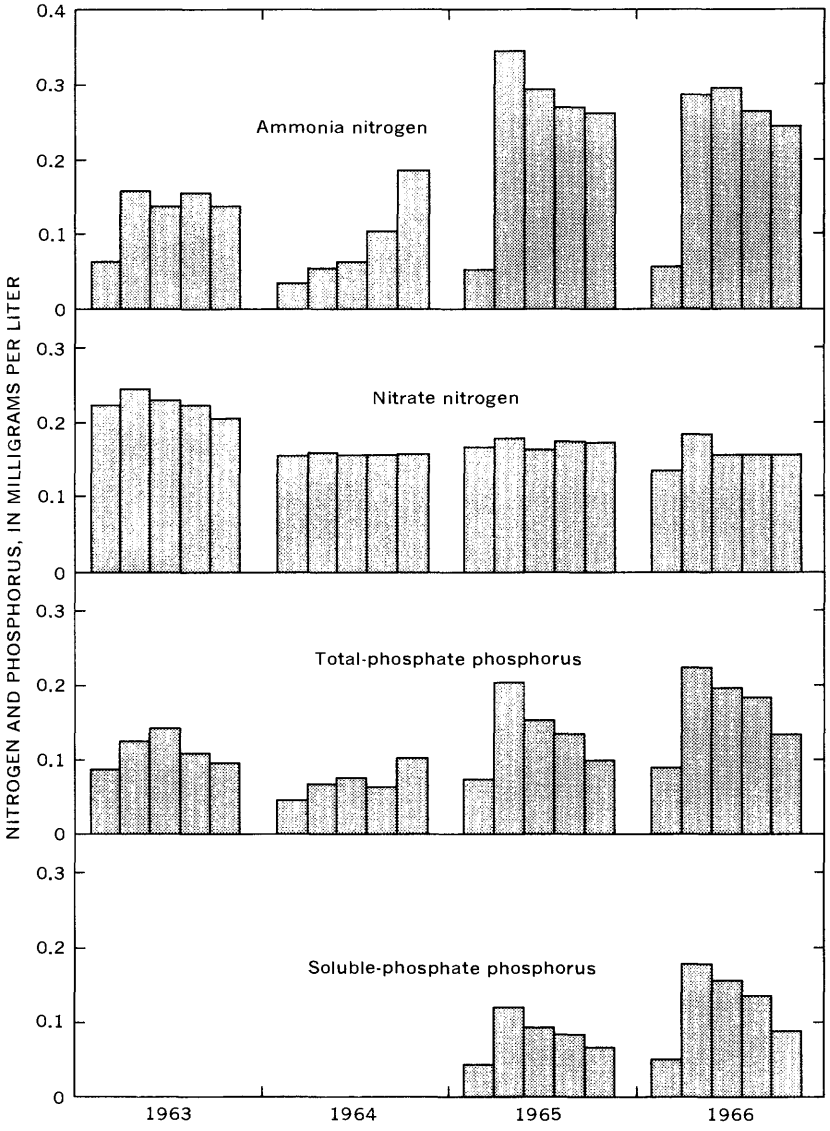


FIGURE 8.—Downstream changes in annual mean concentrations of ammonia nitrogen, nitrate nitrogen, total-phosphate phosphorus, and soluble-phosphate phosphorus during 1963–66. Each group of five bars represents sampling stations arranged in downstream order from left to right as follows: 21.0, 12.6, 10.4, 7.7, and 1.9. Water samples were collected 1 m below stream surface. Annual mean concentrations are weighted by time.

interaction might be due to a trend toward higher total phosphate downstream and lower concentrations upstream in 1964 (fig. 8). This trend differs from the pattern of concentration in 1965–66; after effluent was introduced, concentrations were highest at the first station below the outfall (12.6) and decreased progressively downstream. The differences between the means for the two periods (1963–64 and 1965–66) may not be great enough to mask the interacting effect of the unusual downstream trend of higher total phosphate in 1964 (fig. 8).

TABLE 2.—*Results of variance analyses comparing downstream changes in mean total-phosphate concentration before and after introduction of effluent from the RTP (cell sizes unequal)*

Source of variance	Sum of squares	Degrees of freedom	Mean square	F value [1]
Before and after first effluent	0. 83	1	0. 83	40. 7
Downstream changes	. 75	4	0. 19	9. 3
Interaction between periods and stations	. 56	4	0. 14	6. 9
Error	13. 09	644	0. 02	
Total	15. 23	653		

[1] Significant at 0.5-percent level using fixed model. Minimum values of F necessary for the indicated probability level and degrees of freedom are:

$$F^{1}_{644\ 0.005} = 7.95; \quad F^{4}_{644\ 0.005} = 3.76.$$

Soluble phosphate was not determined during 1963–64, but concentrations during 1965–66 were usually proportional to total phosphate, and the downstream distribution patterns were similar (fig. 8). Total phosphate exceeds soluble phosphate and orthophosphate in the Duwamish by factors of about 1.5 and 2, respectively.

The variation of nutrient distribution with tide is illustrated by soluble-phosphate concentrations at high and low tide in 1965–66 (fig. 9). The concentrations vary most during the low-flow period at stations 7.7, 10.4, and 12.6, where variation in other tide-influenced hydrographic factors is greatest. The maximum concentrations occur at low tide, partly because sea-water dilution of the RTP effluent and of other fresh-water nutrient sources is minimum then.

NITROGEN

Comparison of annual mean concentrations of ammonia during the period 1963–66 clearly illustrates a nutrient increase in the estuary during 1965–66, following introduction of the RTP effluent (fig. 8).

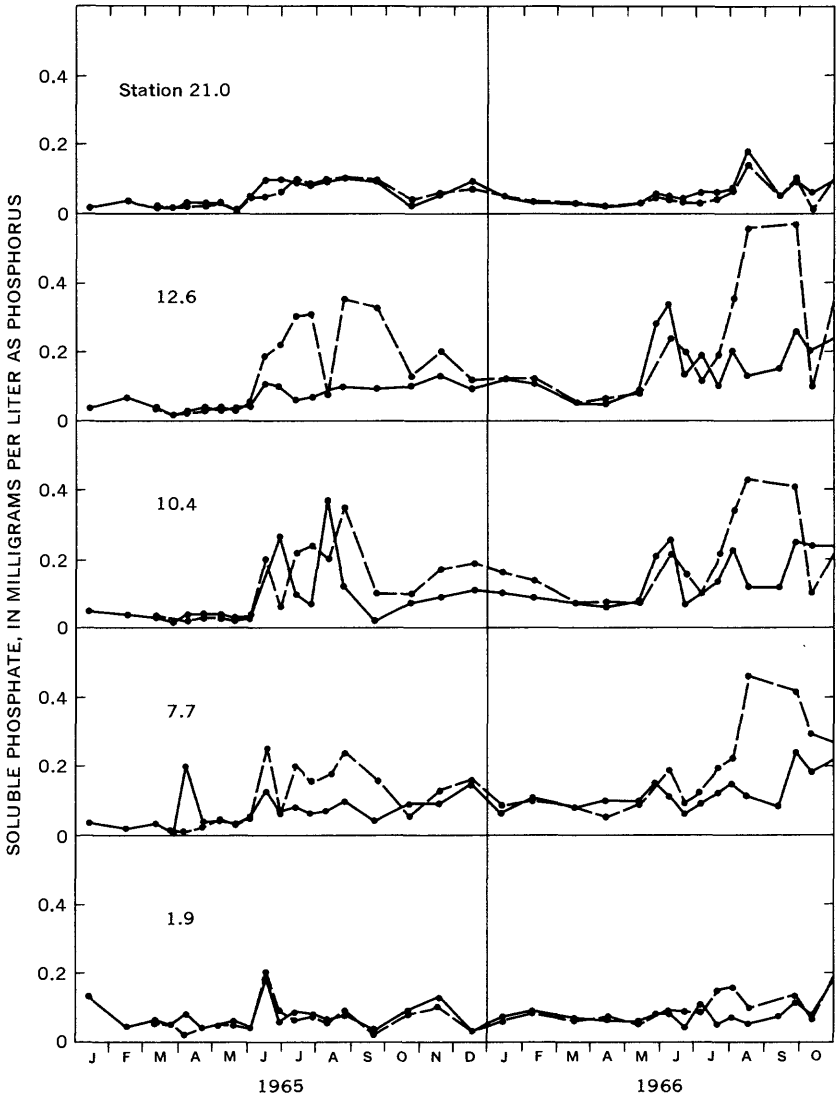


FIGURE 9.—Soluble phosphate at high and low tide (solid and dashed lines, respectively) at five stations January 1965–October 1966. Water samples were collected 1 m below stream surface.

The difference in annual mean concentration of ammonia during the 4 years is only slight at station 21.0, whereas the increase is obvious at stations below the effluent outfall during 1965–66. Nitrate concentrations, on the other hand, did not noticeably change following introduction of the effluent; this was predictable, however, owing to the low nitrate content of the effluent.

Downstream changes in the mean concentration of ammonia before and after introduction of the RTP effluent (1963–64 and 1965–66, respectively) have been compared by variance analysis (table 3). If stations and periods are again considered fixed, the increase in ammonia concentrations after effluent introduction, as well as the concentration differences between stations, is significant at the 0.5-percent level.

TABLE 3.—*Results of variance analyses comparing downstream changes in mean ammonia concentration before and after introduction of effluent from RTP (cell sizes unequal)*

Source of variance	Sum of squares	Degrees of freedom	Mean square	F value [1]
Before and after first effluent	1. 16	1	1. 16	[2] 11. 2
Downstream changes	2. 43	4	0. 61	[2] 5. 8
Interaction between periods and stations	. 73	4	0. 18	1. 8
Error	62. 89	606	0. 10	
Total	67. 21	615		

[1] Minimum values of F necessary for the indicated probability level and degrees of freedom are:

$$F^{1}_{606\ 0.005} = 7.95; \quad F^{4}_{606\ 0.005} = 3.76.$$

[2] Significant at 0.5-percent level using fixed model.

In contrast to total phosphate, there was no significant interaction at the 5-percent level between period and stations for ammonia concentrations, even though the distribution pattern in 1964 of increasing mean ammonia concentrations downstream was similar to, but more pronounced than, that of total phosphate (fig. 8). The greater difference between mean ammonia concentrations before and after effluent introduction than between total phosphate cencentrations before and after effluent introduction, may have masked the interaction effect of ammonia between stations and periods.

MAXIMUM BIOMASS PRODUCED

In vitro nutrient bioassays suggest that the addition of effluent stimulates phytoplankton production. Therefore, an increase in the maximum biomass of the bloom might result from the addition of effluent, even though the timing of the bloom is not altered. Figure 10 shows results of carbon-14 assimilation by a mixed phytoplankton culture in filtered river water with and without filtered RTP effluent added. A green alga (*Scenedesmus* sp.) was the most abundant organism in both culture experiments.

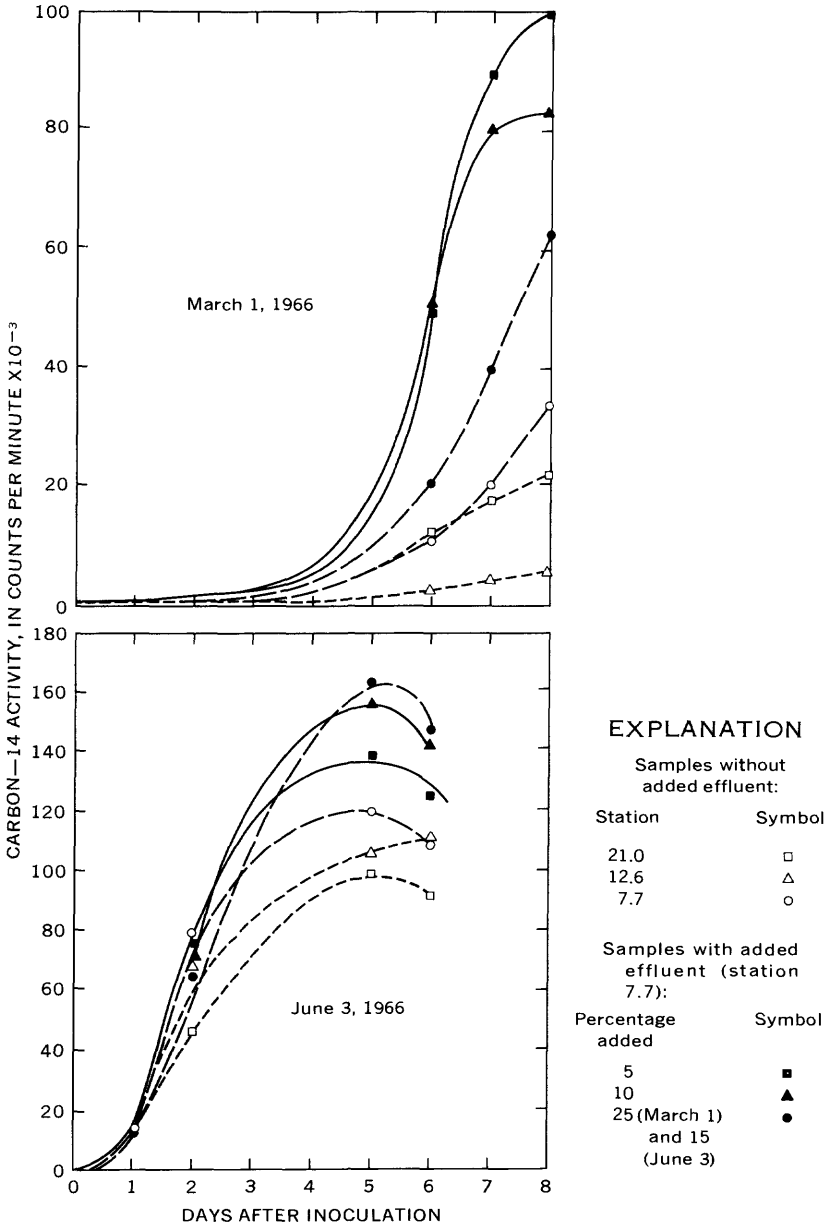


FIGURE 10.—Increase in assimilation of carbon-14 indicates in vitro response of phytoplankton to added effluent nutrients.

Assimilation was greatest in water from station 7.7 with effluent added, but only in the June 3 experiment was the amount assimilated proportional to the amount of effluent added, in fact, the assimilation order was reversed in the March 1 experiment. In general, assimilation in water without added effluent increased downstream in samples collected June 3, 1966, but a progressive downstream increase in assimilation was not apparent in the March 1 experiment because water from station 21.0 (without added effluent) produced a greater biomass than water from station 12.6. Nutrient concentrations, final carbon-14 activity, and some comparable cell concentrations from the March 1 experiment are given in table 4. Of course, trace elements and various organic growth stimulants, which were not determined, may account for some of the variability not explained by nitrogen and phosphorus compounds. Nevertheless, effluent addition does stimulate the assimilation rate of a green algal species (*Scenedesmus*) in fresh water and in dilute sea water (specific conductance about 7,000 micromhos). Unsuccessful attempts were made to grow pure cultures of a marine diatom (*Skeletonema costatum*) that is found in the blooms in low-salinity estuarine water under the stated artificial conditions.

Annual mean concentrations of chlorophyll *a* in 1965 and 1966 were compared to determine whether the increase in mean effluent discharge from 34,000 m^3/day in 1965 to 49,200 m^3/day in 1966 caused a similar increase in total biomass. The chlorophyll concentrations in 1965 and 1966 were not significantly different at the 10-percent confidence level, but station-to-station differences were significant at the 5-percent level

TABLE 4.—*In vitro carbon-14 assimilation by phytoplankton inoculated into filtered river water containing different effluent nutrient contents*

[Water samples were collected Mar. 1, 1966]

Station	Effluent added (percent)	Initial nutrient concentrations		Final C-14 activity (counts per minute, in thousands)[1]	Final number of cells per milliliter (thousands)
		Ammonia plus nitrate (mg N/l)	Phosphate (mg P/l)		
7.7	25	4.0	1.8	62	----------
7.7	10	1.8	.77	83	----------
7.7	5	1.1	.43	99	50
7.7	0	.38	.08	34	12
12.6	0	.32	.05	5.4	3.7
21.0	0	.08	.05	21	----------

[1] 8 days after inoculation.

(table 5). Statistically, the interaction between stations and years was not significant. The comparison of mean chlorophyll for 1965 and 1966 shows, therefore, that the 46-percent effluent increase in 1966 did not increase phytoplankton production in the estuary. However, sampling frequency during the bloom periods may not have been sufficient to detect possible differences in the annual mean concentration. Maximum gross productivity in 1966 (4,680 mg C/m²/day) was greater than that in 1965 (3,740 mg C/m²/day), and maximum chlorophyll *a* concentrations were 70 and 28 mg/m³ during 1966 and 1965, respectively. Although these data indicate that phytoplankton production did increase in 1966 compared to 1965, the observations themselves do not indicate this; however, the observations may not represent the production maxima that actually occurred during the 2 years. More frequent sampling during the bloom period in 1965 might have shown a higher maximum.

TABLE 5.—*Results of variance analyses comparing downstream changes in mean annual chlorophyll a concentrations in 1965 and 1966 (cell sizes unequal)*

[Mean effluent discharge averaged 34,000 m³/day in 1965 and 49,000 m³/day in 1966.]

Source of variance	Sum of squares	Degrees of freedom	Mean square	*F* value [1]
1965 versus 1966	0. 51	1	0. 51	0. 039
Downstream changes	130. 34	4	32. 59	[2] 2. 49
Interaction between periods and stations	7. 36	4	1. 84	0. 14
Error	3, 288. 20	251	13. 10	
Total	3, 426. 41	260		

[1] Minimum values of *F* necessary for the indicated probability level and degrees of freedom are:

$$F^{1}_{251\ 0.10} = 2.73; \qquad F^{4}_{251\ 0.05} = 2.42$$

[2] Significant at 5-percent level using fixed model.

EFFECTS OF PHYSICAL FACTORS

Seasonal and year-to-year variations of incident light intensity and estimated residual intensity at a depth of 2 m are shown in figure 11. Total insolation during August of each year was about the same (13.5×10^3, 12.8×10^3, and 15.5×10^3 langleys in 1964, 1965, and 1966, respectively). A comparison of incident and residual light indicates that conditions in August 1964 would probably have been sufficient for a phytoplankton bloom to develop if other environmental factors also had been favorable. The relation between light penetration and the depth and extent of mixing in the surface-water layer probably has an important influence on phytoplankton production because the depth

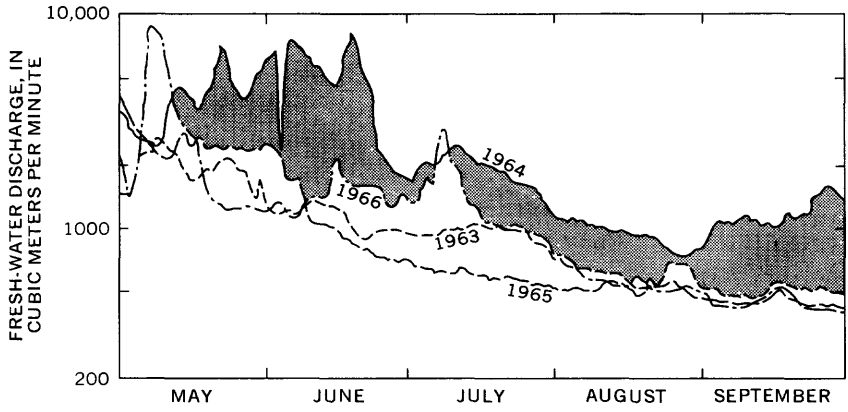


FIGURE 12.—Mean daily fresh-water discharge at station 21.0, May-September 1963–1966. Discharges in 1964 that exceeded those of 1963 and 1965–66 are emphasized by shading.

Further evidence of the effect of these hydrographic factors on the timing of blooms is shown by the relation of the chlorophyll *a* concentration that was determined at station 7.7 to fresh-water discharge and tidal-prism thickness during the summers of 1965 and 1966 (fig. 13). During both years, the blooms occurred when the volume of discharge and the tidal-prism thickness were at their minimums; this timing indicates that water-retention time and stability in the photic zone were probably at their maximums. These conditions apparently promoted rapid phytoplankton growth by retaining organisms in the zone of optimum light intensity within a few meters of the surface. Thus, net production was high because the organisms were not carried below the compensation depth (usually between 2 and 3 m) into zones where respiration exceeds photosynthesis. The standing stock thereby increased rapidly under stratified conditions, as a result of the increased net production.

The occurrence of low discharge earlier in 1965 than in 1966 may partly explain the more gradual increase of the standing stock in 1965. Discharge remained relatively high during the early summer of 1966, but declined rapidly and reached the 550-m^3/min level during the first week in August, the week having the most favorable tides. Thus, conditions became ideal for bloom development rather suddenly in 1966, and a sudden outburst resulted.

The stability of the photic zone at station 7.7 increased with decreasing discharge, as indicated by profiles of temperature and specific conductance for June–August 1966 (fig. 15). Stability seemed to be greatest when the tidal-prism thickness and discharge approached minimums in early August. The estuary was stratified earlier, as shown by

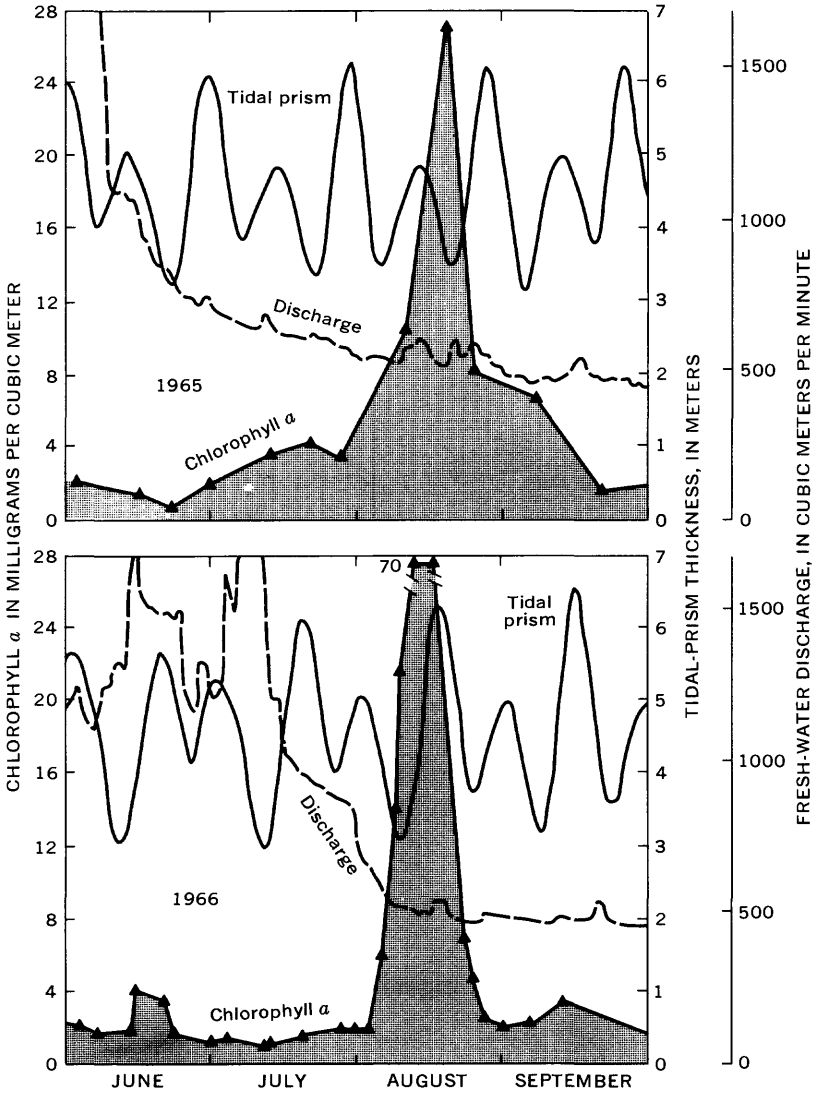


FIGURE 13.—Relation of phytoplankton-bloom timing to fresh-water discharge and tidal-prism thickness, June–September 1965 and 1966. Blooms are indicated by an abrupt increase in chlorophyll *a* concentration.

the profiles in June (fig. 14), but turbulent mixing apparently was strong in the upper 3–4 m. In August, the decreased river discharge and increased surface temperature resulted in stability of even the upper 1–2 m. Apparently this stability is necessary to enable photosynthesis to exceed respiration sufficiently to promote the growth of

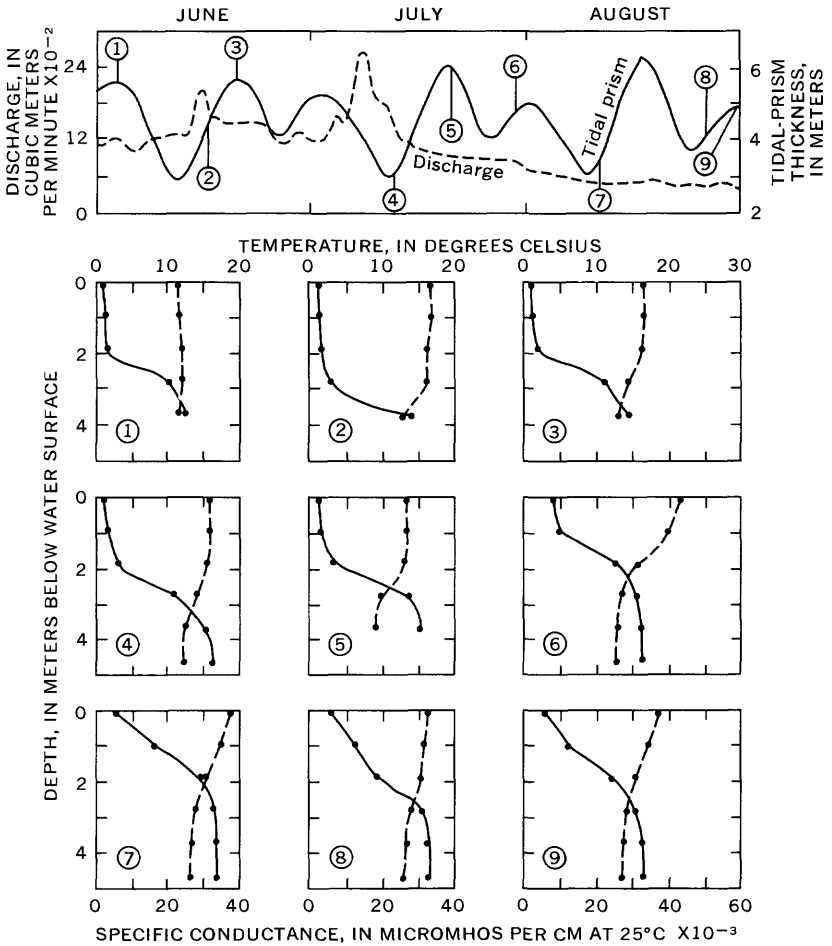


FIGURE 14.—Vertical profiles of specific conductance (solid line) and temperature (dashed line) at station 7.7 near low tide during June–August 1966 in relation to fresh-water discharge and tidal-prism thickness.

a bloom. The maximum observed differences of specific conductance and temperature between the surface water and water at a depth of 3 m occurred August 11 when tidal-prism thickness was at a minimum and discharge was 550 m^3/min. The fact that these conditions coincided with maximum phytoplankton productivity (figs. 4, 13) strongly sug-

gests that the factors affecting stability and retention time also indirectly control the timing of phytoplankton blooms.

Conditions of low fresh-water discharge and minimum tidal-prism thickness occurred again during September of 1965 and 1966, but blooms did not occur. During both those years, however, incident light and temperature decreased greatly in September compared to the prebloom levels of about August 1 (fig. 12). Although conditions of discharge and tide were favorable during the two Septembers, light intensity may not have been sufficient to promote the photosynthetic rate necessary for a bloom to develop or to raise the temperature of the surface water high enough to maintain stability.

Control of the timing of phytoplankton blooms by physical factors was further indicated by comparing in situ chlorophyll *a* concentrations with the "potential" concentrations cultivated in water collected on the same date but incubated under artificial conditions (fig. 15). The increases in biomass that were grown under artificial conditions during short periods before and after the bloom indicate that the water could have supported growth. Thus, the lack of bloom then suggests that the physical environment in situ was not suitable to sustain the growth over a longer period. The failure of "potential" chlorophyll *a* concentrations to exceed in situ values during the bloom was probably the result of suboptimal intensities of artificial light.

Conditions of reduced vertical turbulence and greater stability, which occur during periods of minimum tidal-prism thickness and minimum discharge, are also indicated by greater minimum specific conductance near the surface at station 7.7 (fig. 4). Maximum specific conductances do not change appreciably with tidal-prism thickness, so periods of thin prism thickness are characterized by greater minimum conductivities as well as less diurnal change in specific conductance. If light intensity is great during these periods, surface temperatures will increase. According to Riley (1942), vertical differences in temperature are due to the combined effect of heating by solar radiation and submergence of warmer surface water by vertical turbulence; therefore, the difference between surface- and bottom-water temperatures (Δ°C) during the year indicates the extent of stratification or vertical turbulence. Adequate data are not available for the more precise variables that describe stratification and retention time; therefore, the importance of these two factors in controlling phytoplankton productivity is examined using a multiple regression analysis with Δ°C and

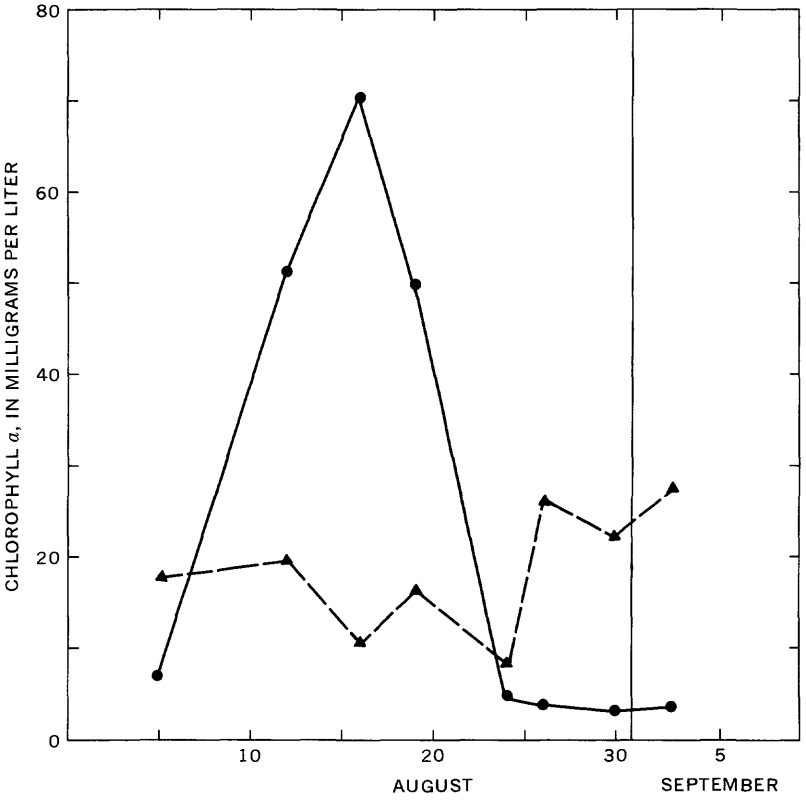


FIGURE 15.—Contrast between growth of phytoplankton in place (solid line) and "potential" phytoplankton (dashed line) during August and early September 1966. Both types of samples were collected at low tide 1 m below stream surface at station 7.7. Growth is indicated by chlorophyll *a* concentrations. "Potential" concentrations were determined after 5–6 days of static incubation under artificial conditions (light intensity about 7,000 lux, temperature about 20°–21°C).

fresh-water discharge as independent variables and gross productivity as the dependent variable. Fourteen monthly or biweekly observations from May to October during 1965–1966 were used in the analysis. The temperatures were measured biweekly, usually about a week before productivity measurements. The Δ°C values are the difference between the mean of high- and low-tide surface determinations and the mean of high- and low-tide bottom determinations. The computed prediction equation for the relationship is

$$\log \hat{Y} = 4.113 + 0.243X_1 - 0.729 \log X_2,$$

where $\hat{Y}$ is gross productivity, in milligrams carbon per square meter

per day; X_1 is Δ°C (difference between surface and bottom water temperature); and X_2 is discharge, in cubic meters per minute. Correlation coefficient, 0.71 for temperature change (Δ°C) only and 0.78 for both Δ°C and discharge together are significant at the 1-percent level. The upper and lower 95-percent confidence intervals for the mean productivity are 610 and 222 mg C/m^2/day, respectively. Temperature and discharge presumably accounted for 61 percent of the variation in productivity, even though they were not specific measurements of stability and retention time.

RELATION OF PHYTOPLANKTON TO DISSOLVED OXYGEN

DISTRIBUTION OF DISSOLVED OXYGEN

Minimum concentrations of DO are present near the bottom in the saline-water wedge between stations 7.7 and 10.4 during periods of minimum fresh-water discharge and of minimum tidal-prism thickness. The available determinations of daily minimum DO in samples from less than 1 m above stream bottom at station 7.7 are illustrated in figure 16. Minimum concentrations of DO observed during pre-effluent conditions in 1963 were nearly as low as those observed during effluent conditions in 1966; however, concentrations remained below 3.0 mg/l for a longer period in 1966 than in 1963. Summer DO data from 1965 are inadequate because the automatic monitoring equipment at station 7.7 was inoperative. Nevertheless, limited data from manual sampling show concentrations below 3.0 mg/l at that station during late August and early September.

During the summer of 1964, when fresh-water discharge was never less than 760 m^3/min, observed concentrations of DO were below 3.0 mg/l on only 2 days and were considerably greater than 3.0 mg/l during most of the summer.

The timing and duration of minimum DO concentrations are greatly dependent on fresh-water discharge and tidal-exchange conditions. The cyclic nature of minimum concentrations in 1963 and 1966 (fig. 16) is directly related to tidal-prism thickness, whereas the long-term trend of the minimum concentration within each cycle seems to be related to discharge. Lowest minimum concentrations usually are not reached until discharge falls below about 600 m^3/min. Thus, minimum concentrations remained relatively high in 1964 when discharge was always greater than 760 m^3/min.

The minimum DO concentration also seems to be related to the occurrence of phytoplankton blooms. Very low DO values followed blooms during the summers of 1963, 1965, and 1966 (fig. 16). In contrast, daily DO minimums remained relatively high during the summer

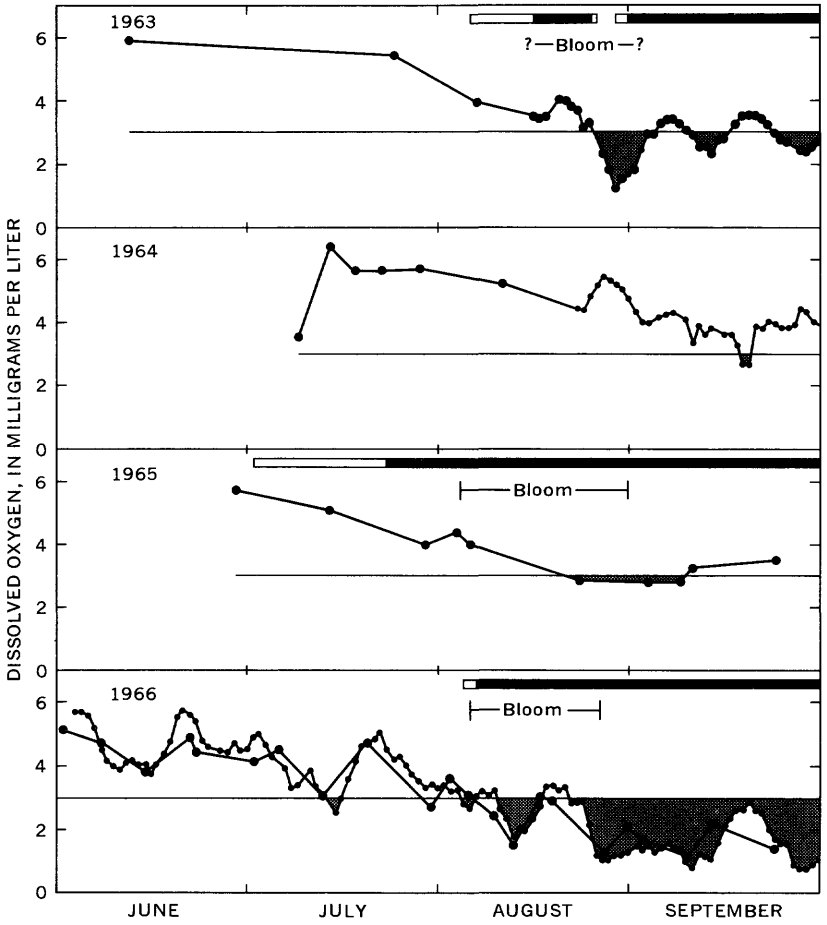


FIGURE 16.—Relation of minimum DO concentrations at station 7.7 to phytoplankton-bloom timing and to fresh-water discharge, June–September 1963–66. Water samples were collected during low tide less than 1 m above stream bottom. The minimum concentrations were usually recorded during continuous monitoring throughout a tidal cycle. Method of sampling was as follows: Manual, large circle; automatic, small circle. Fresh-water discharge is indicated as follows: Less than 600 m^3/min, solid horizontal bar; 600 to 760 m^3/min, open bar; more than 760 m^3/min, no bar. Approximate periods of phytoplankton bloom are based on chlorophyll *a* concentrations greater than 4.0 mg/m^3. Dissolved-oxygen concentrations less than 3 mg/l are emphasized by shading.

of 1964, when there was no bloom. Further, the relationship between phytoplankton biomass and minimum DO is inverse in water samples collected less than 1 m above stream bottom from an eight-station series (between kilometers 1.9 and 10.4) throughout the summer of 1966 (figs. 17, 18). Maximum concentrations of chlorophyll *a* in the estuary

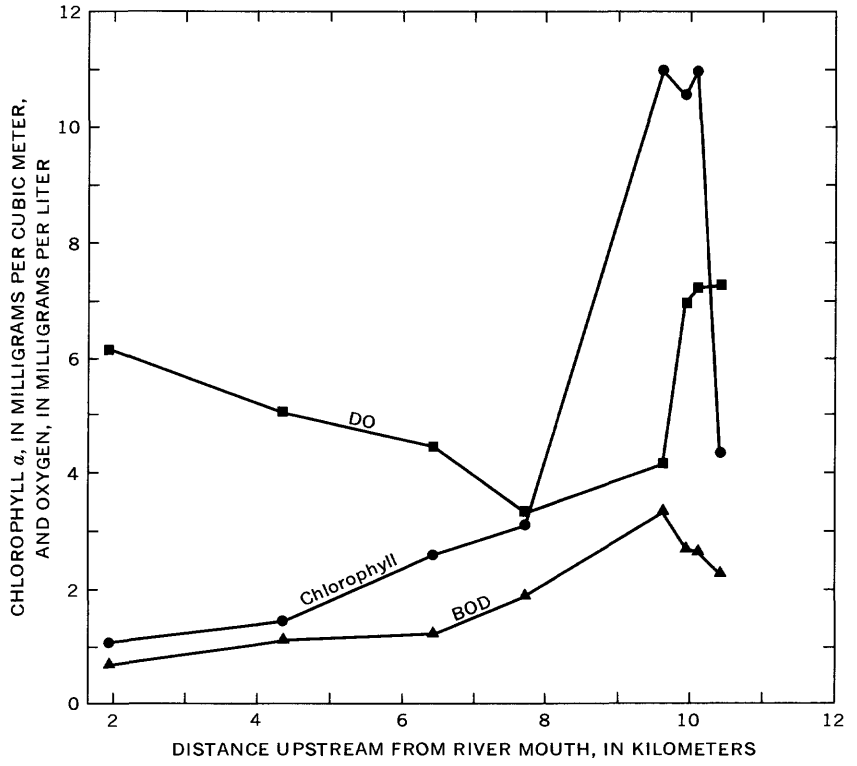


FIGURE 17.—Longitudinal distribution of chlorophyll *a*, BOD, and DO in the estuary at low tide. Water samples were collected less than 1 m above stream bottom during June–September 1966. Number of observations: 22 at station 7.7 and 14 each at remaining stations. Mean concentrations are weighted by time.

consistently occur between the point of minimum low-tide DO (station 7.7) and the point of minimum high-tide DO (station 10.4 or above).

Concentrations of DO in the saline-water wedge decrease during late summer apparently because retention time of that water increases as discharge and consequent flushing rate decrease. Water in the part of the wedge that is farthest upstream has been retained in the estuary for the longest time without reaeration. This is true in a salt-wedge estuary because there is a net upstream movement of bottom water, and because the only escape for that water is through mixing and entrainment within the brackish surface-water layer. As retention time of the bottom water increases, the DO concentration decreases because the BOD has a longer time to act.

The timing of the DO minima is related to fresh-water discharge and tidal-prism thickness, as is the timing of phytoplankton blooms.

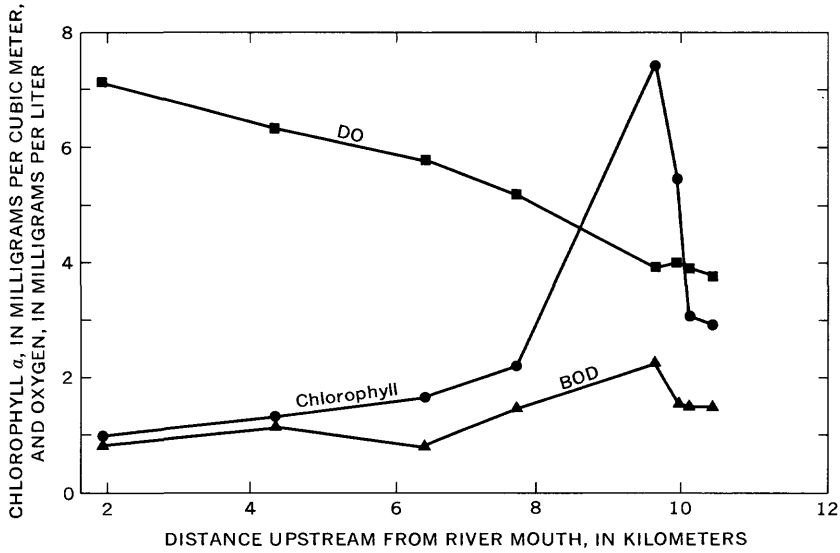


FIGURE 18.—Longitudinal distribution of chlorophyll *a*, BOD, and DO in the estuary at high tide. Water samples were collected less than 1 m above stream bottom during June–September 1966. Number of observations: 20 at station 7.7 and 11 each at remaining stations. Mean concentrations are weighted by time.

The similar timing of blooms and minimum DO with hydrographic conditions might suggest that these two events are not directly related to each other. A decrease in oxygen concentration, however, is necessarily caused by an oxygen demand, and phytoplankton organic matter serves as a possible source for an important part of the total in situ BOD.

RELATION OF PHYTOPLANKTON TO BIOCHEMICAL OXYGEN DEMAND AND DISSOLVED OXYGEN

In the estuary, the longitudinal relation between BOD, chlorophyll *a*, and DO indicates that the BOD contributed by phytoplankton is consistently greatest in the area of minimum DO. Figures 17 and 18 show that the maximum mean BOD determined in the saline-water wedge less than 1 m above stream bottom occurred at station 9.7 at both high and low tide, and that maximum concentrations of chlorophyll *a* usually coincide with maximum BOD. In contrast, the minimum DO concentrations determined at low tide usually occurred at station 7.7, and the minimum high-tide concentrations occurred at station 10.4. Station 10.4 was the point farthest upstream at which samples were collected during these studies; hence, even lower oxygen concentrations might have been present above this station at high tide.

However, no such concentrations were detected by the automatic monitor at station 12.6 farther upstream. The relationships depicted in figures 17 and 18 show that between stations 1.9 and 10.4 in the lower estuary, BOD and chlorophyll *a* are related to each other, and each is negatively correlated with DO. Also, the water with maximum concentrations of BOD and chlorophyll *a* usually occurs at station 9.7 regardless of tide stage.

The part of the estuary where the maximum concentrations of BOD and chlorophyll *a* occur near the bottom is a transitional area between the shallower upriver reach and the widened and dredged lower reach. Extensive organic deposition occurs in this transition area. This is indicated by the relatively greater oxygen demand of bottom deposits in the transitional area compared to that of deposits both upstream and downstream (G. W. Isaac, Metro, written commun., 1964). The great number of bloom-causing diatoms in the particulate matter in water samples collected less than a meter above stream bottom at stations 7.7 and 9.7 following phytoplankton blooms also indicates the deposition of organic material. A sample collected near the bottom at station 7.7 on August 10, 1965, had a chlorophyll *a* concentration of 4.6 mg/m^3 and a total cell number of 3,900 per milliliter. *Skeletonema costatum* was abundant in the surface water at this station and comprised 22 percent of the cells in the bottom water. A chlorophyll concentration of 5.5 mg/m^3 was determined in a bottom-water sample collected from station 9.7 on August 26, 1966. Cells in the sample totaled 11,300 per milliliter, of which *Thalassiosira* comprised 40 percent. These data indicate that particulate matter from phytoplankton blooms near the surface becomes an important component of the highly organic bottom deposits in this area.

Figure 19 shows the seasonal relationships between concentrations of chlorophyll *a*, BOD, and DO at low tide in the area between stations 7.7 and 10.0. Although chlorophyll and BOD increased slightly in June 1966, the maximum concentrations occurred during the bloom in August. The seasonal relationship is also indicated by concentrations of chlorophyll *a* and BOD in the reach between stations 7.7 and 10.0. Mean concentrations of chlorophyll *a* and BOD in this section of the estuary were greatest during August, when the bloom occurred (fig. 20). The seasonal timing of maximum chlorophyll *a* and BOD in the saline-water wedge, as well as their correlated distribution throughout the estuary, strongly indicates that the organic matter contributed by sinking phytoplankton cells during blooms is an important source of BOD in the Duwamish.

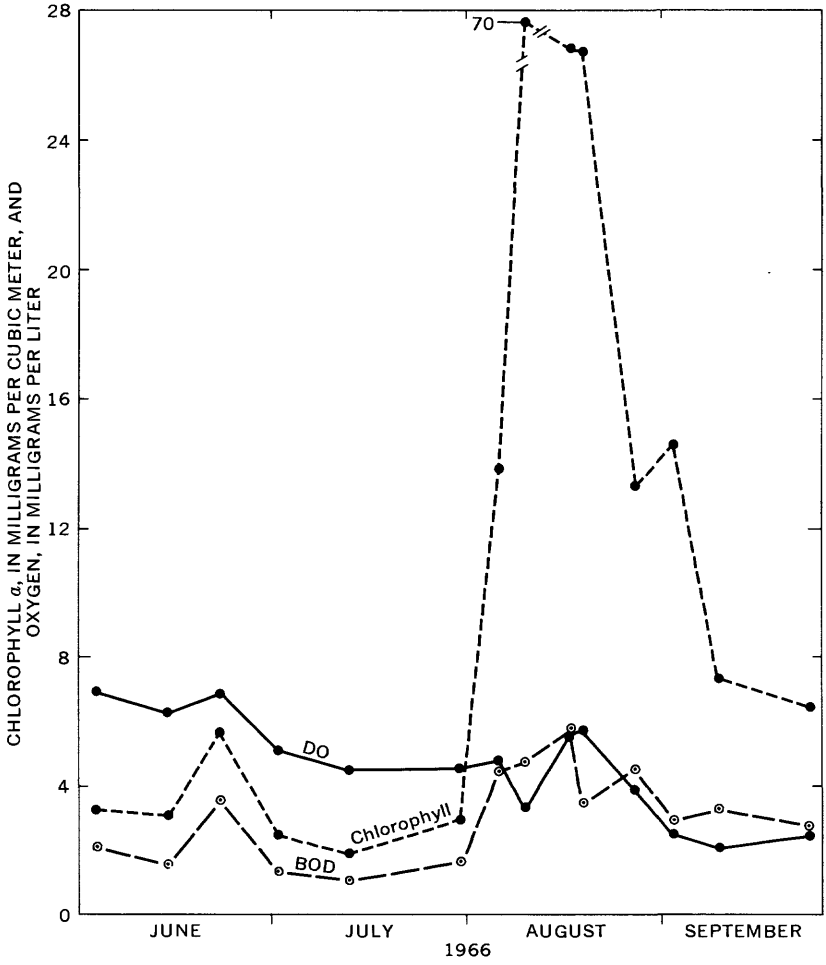


FIGURE 19.—The seasonal variation of chlorophyll *a*, BOD, and DO in the area of minimum DO during the summer of 1966. Water samples were collected at low tide less than 1 m above stream bottom at stations 10.0, 9.7, and 7.7. Each point on graph is a mean value for the three stations.

A pronounced decrease in DO, to concentrations less than 1.0 mg/l, occurred in the bottom water at stations 7.7 and 9.7 in late August 1966. The decrease closely followed the observed maximum concentrations of BOD and chlorophyll *a* (figs. 16 and 19). Part of the decrease may have been initiated by hydrographic conditions, because it coincided with a period of minimum tidal-prism thickness. However, the DO minimums were only slightly greater during the period of thicker tidal prism in early September; this fact suggests that the

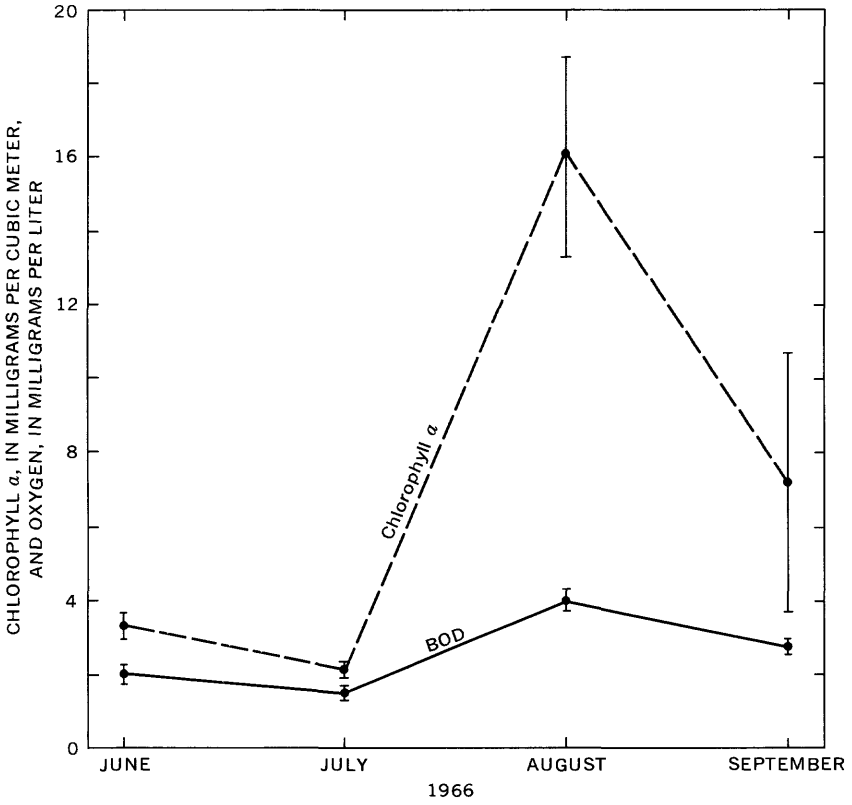


FIGURE 20.—Monthly means of chlorophyll *a* and BOD in the area of minimum DO, June–September 1966. Water samples were collected at low tide less than 1 m above stream bottom at stations 10.0, 9.7, and 7.7. Each point on graph is a monthly mean (weighted by time) for the three stations. Vertical bars indicate standard error.

increased BOD was contributing significantly to DO depression. Furthermore, minimum DO concentrations at the start of the August bloom were considerably higher during the period of thin prism, about August 11–15, than they were during a similar period in late August and early September. This was true even though fresh-water discharge was about the same during both periods. The minimum DO concentration increased in response to hydrographic conditions about September 20, when the tidal prism was thickest, before dropping again to low levels in late September. The timing and persistence of DO is largely related to discharge and tidal-prism thickness, both of which affect water-retention time. However, increased BOD from the bloom seems to interact in this relationship to some extent and

produces lower and more persistent DO minimums than might otherwise be expected during conditions of low discharge and tidal action.

The increases in chlorophyll *a* concentration and BOD during August 1966 were not of the same magnitude (fig. 19). As previously suggested, the disproportionately lower BOD values could be due to the dependence of oxygen demand rates on DO concentration. Also, a large proportion of cells were living during this period, and the BOD of living cells probably would be less than that of dead cells, since only a small part of the cell carbon would be available for oxidation. Conversely, when the concentration of chlorophyll *a* decreased following the bloom, more of the cells probably were dead; thus suggesting a relatively high BOD in a low-chlorophyll sample could actually be attributed to organic matter from phytoplankton cells that were dead and therefore lacked chlorophyll. If some of the chlorophyll *a* were destroyed by decomposition, the amount present would not constitute a valid measure of oxidizable cell carbon (BOD).

A close proportional relationship between chlorophyll *a*, BOD, and DO is shown by a vertical profile of samples collected at station 9.7 during the bloom in 1966 (fig. 21). The profile also illustrates the significance of the phytoplankton bloom as a source of BOD. The amount of oxygen produced by this large biomass of phytoplankton can be reversed to indicate the amount of oxygen consumed by respiration and decomposition when the biomass sinks below the photic zone. The sharp stratification shows that the productive zone was limited primarily to the upper 2 m.

A comparison of all low-tide observations of chlorophyll *a* and BOD throughout the estuary showed that the relationship between these variables was curvilinear, that is, high concentrations of chlorophyll *a* were not accompanied by proportionately high BOD values, but transformation of the data into logarithms resulted in linearity (fig. 22). The equation for the calculated line is

$$\log \hat{Y} = 0.015 + 0.46 \log X,$$

where $\hat{Y}$ is the predicted BOD concentration and X is the chlorophyll *a* concentration. The coefficient of correlation, 0.82, is highly significant, and the upper and lower 95-percent confidence limits for the mean BOD concentration are, respectively, 1.9 and 1.6 mg/l. The interception of the regression line with the ordinate (BOD) indicates that other BOD sources exist in addition to phytoplankton cells containing chlorophyll *a;* as noted earlier, one source may be dead

cells without chlorophyll *a*. This analysis shows the importance of phytoplankton organic matter as a source of BOD in the estuary.

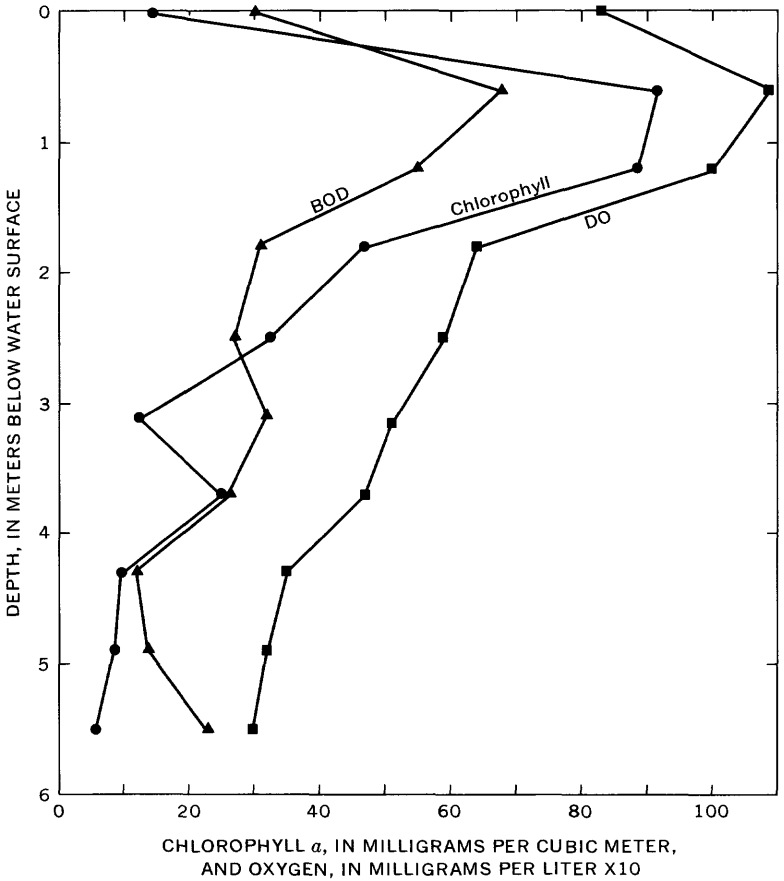


FIGURE 21.—Vertical distribution of chlorophyll *a*, BOD, and DO at station 9.7 on August 9, 1966, during the phytoplankton bloom.

EVALUATION OF RESULTS

TIMING OF PHYTOPLANKTON BLOOMS

Concentrations of ammonia and phosphate increased significantly in the Duwamish estuary during 1965–66, following introduction of

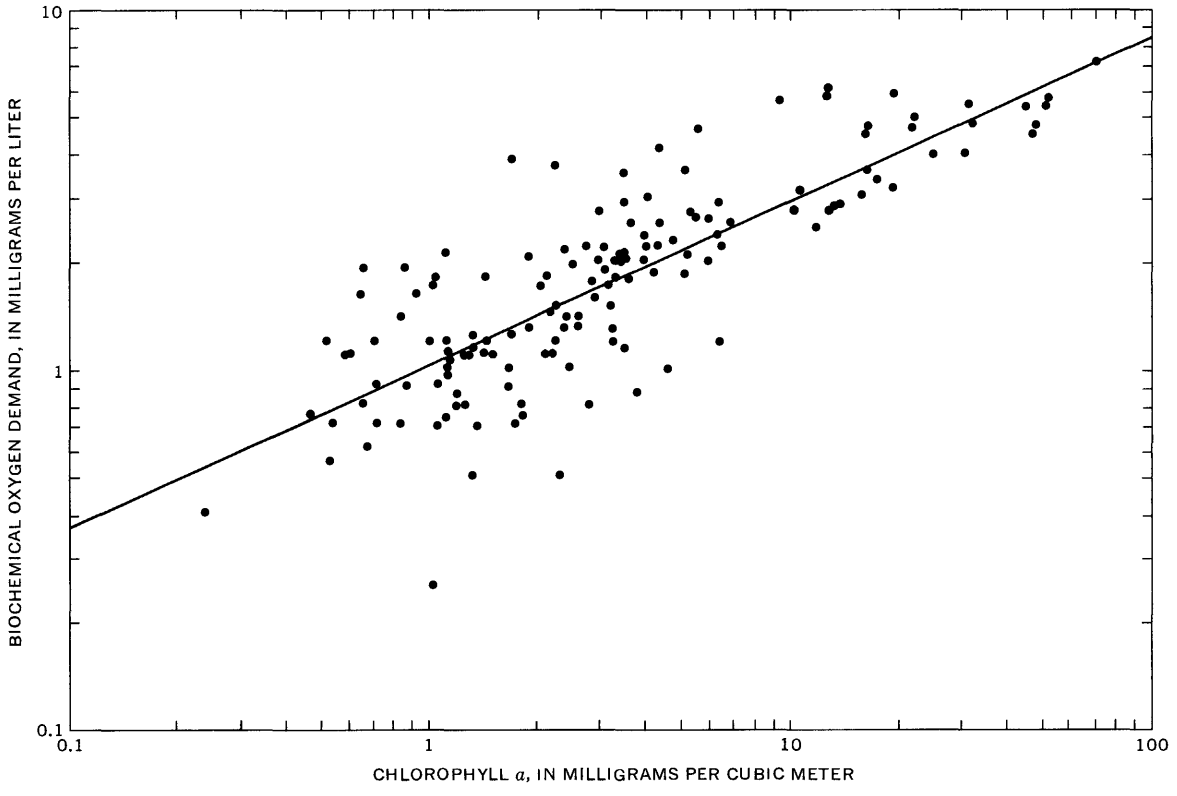


FIGURE 22.—Logarithmic relationship of chlorophyll *a* to BOD during the summer of 1966. Samples were taken at low tide less than 1 m above stream bottom at eight stations (1.9 through 10.4) and at stream surface at two stations (1.9 and 7.7).

the RTP effluent, and blooms occurred in the lower estuary in August of both years, during the period of maximum nutrient concentrations. In contrast, no bloom occurred in 1964, when nutrient concentrations were comparatively low before the introduction of effluent. The maximum chlorophyll *a* concentrations observed during each of the 3 years (1964–66) were 3, 28, and 70 mg/m^3, respectively.

Considering only the summer periods, the lowest mean concentration of total phosphate during 1964–66 (0.10 mg P/l) occurred in 1964, and it coincided with the minimum concentration of chlorophyll *a* (3 mg/m^3). The mean quantities of soluble phosphate and orthophosphate during that summer may have been about 0.07 and 0.05 mg P/l, respectively; this figure is based on an average ratio among the three phosphate forms in the Duwamish.

The comparison of nutrient concentrations and phytoplankton activity during 1964–66 suggests that the blooms in 1965–66 resulted from nutrients added by the RTP effluent. The addition of nutrients, however, is probably not the sole cause; the following points also deserve consideration:

1. Mineral nutrient concentrations during 1964 were relatively high compared to concentrations in other marine areas which support large blooms.
2. Nutrient concentrations were greater at station 7.7 during blooms in 1965 and 1966 than during a similar period in 1964, yet the blooms declined in both 1965 and 1966. Nutrients probably were not limiting during the blooms: there was no apparent reduction in situ in nutrient concentration that was related to phytoplankton growth, and the assimilation rate per unit chlorophyll *a* was high, as shown by the steep slope of the productivity-chlorophyll *a* regression line (fig. 2).
3. In spite of relatively high nutrient concentrations during most of the 1965–66 summer periods (fig. 7, 9), phytoplankton blooms occurred only during August of each year.
4. No appreciable change in phosphate concentration was apparent during 1964–66 at station 1.9 (figs. 7, 8, and 9), yet phytoplankton blooms occurred there in 1965 and 1966, but not in 1964.
5. The occurrence of supersaturated oxygen in August 1963 indicated the presence of bloom activity prior to completion of the RTP.

The phosphate concentrations in the Duwamish during 1964 probably exceeded quantities that would limit the production of phytoplankton in most similar estuaries. Because the effect of various nutrient concentrations on in situ production under constant physical conditions is not known in the Duwamish, the possibility that limiting nutrient concentrations are higher there than in other estuaries should be considered. A lower limiting concentration in the Duwamish, however, would seem more reasonable because of the relatively great turbulence and exchange rate of the Duwamish water; these two factors would tend to increase the effective contact time between existing nutrients and phytoplankton cells. Therefore, comparison with the nutrient content in other productive but probably less turbulent estuaries seems justified as one approach to evaluating the possible limiting effect of nutrient concentrations in the Duwamish.

Because relatively large phytoplankton blooms develop in other estuaries with nutrient contents at least as low as those in the Duwamish, the difference between the phytoplankton standing stock in the Duwamish before and after effluent introduction can not be attributed solely to the difference in nutrient concentrations. Pratt (1965) has described diatom blooms in Narragansett Bay, Rhode Island, in which *Skeletonema costatum* frequently exceeded 50,000 cells per milliliter. Inorganic phosphate concentrations there ranged between 0.03 and 0.05 mg P/l at the beginning of rapid phytoplankton growth, but reached very low levels, along with nitrate and silicate, at the bloom maximum. In describing the nutrient distribution in Raritan Bay, Jeffries (1962, p. 24) showed that the seasonal mean concentrations of inorganic phosphate ranged from 0.06 to 0.14 mg P/l. Maximum chlorophyll *a* concentrations in excess of 100 mg/m^3 are also reported from Raritan Bay (Patten, 1961, p. 372). According to Patten, Mulford, and Warinner (1963, p. 5, 7), chlorophyll *a* reached a maximum concentration of 22 mg/m^3 in Lower Chesapeake Bay, where inorganic phosphate ranged from 0.07 mg P/l to barely detectable. The mean concentrations at all stations ranged from 0.007 to 0.12 mg/l, but the authors found no clear relationship between nutrients and abundance of phytoplankton. The inorganic phosphate concentrations in the English Channel were comparatively low at the beginning of the spring bloom, ranging between 0.014 and 0.022 mg P/l (Raymont, 1963, p. 148). Thus, nutrient concentrations in the Duwamish during 1964 were high (at least 0.10 mg/l) compared to those necessary for development of large phytoplankton blooms in other estuaries.

Other evidence also suggests that nutrient concentrations in 1964 were adequate for development of a bloom similar to those in 1965 and 1966. That no nutrient depletion was apparent at station 7.7 during

the blooms may be explained in part by the high nutrient concentrations and the relatively constant water exchange and nutrient income, but it also implies that nutrient limitation was not the cause for bloom declines in 1965–66. In estuaries where nutrients are considered to limit production, the nutrient concentrations decrease greatly and approach zero at the bloom maximums (Pratt, 1965, p. 174, 175). In the absence of a nutrient reduction during the blooms in 1965 and 1966, the production probably should have continued to increase rather than decline, if in fact the nearly two-fold increase in nutrient concentrations after effluent introduction was the sole cause of bloom development.

The high assimilation rate per unit of chlorophyll *a* in the Duwamish estuary, as indicated by the steep slope of the regression line for productivity and chlorophyll *a* (fig. 2), also suggests that nutrients were not a limiting factor during the blooms. Stross and Stottlemeyer (1965, p. 139) concluded the same thing in the Patuxent estuary, Maryland, on the basis of similar evidence.

The control of bloom timing other than by nutrient content is further indicated by the following points:

1. Nutrient concentrations were relatively high during most of the low-flow period of 1965 and 1966, yet blooms existed for only about 20 days during August of each year.
2. The sudden outburst in August 1966 caused concentrations of chlorophyll *a* to increase from less than 5 mg/m^3 to more than 30 mg/m^3 in less than 5 days, even though soluble-phosphate concentrations had ranged between 0.1 and 0.2 mg P/l during the two previous months.
3. No appreciable difference in the mean total-phosphate concentration was observed at station 1.2 in the three summers studied (1964–66) in spite of blooms there in 1965 and 1966 but not in 1964.
4. Finally, a phytoplankton bloom probably occurred under pre-effluent nutrient levels during August 1963, as indicated by supersaturated oxygen concentrations in the surface water at station 7.7. All these observations indicate that the nutrient increases attributable to introduction of effluent from the RTP probably was not the primary cause for the blooms in 1965–66.

Hydrographic conditions, rather than nutrient content, apparently control the timing of blooms in the Duwamish. A minimum fresh-water discharge of about 550 m^3/min during a period of minimum tidal exchange and high light intensity increases the photic-zone stability and water-retention time. These conditions in turn favor phytoplankton blooms because the standing stock is apparently held in the shallow photic zone (above the compensation depth), where production greatly exceeds respiration. The compensation depth in the Duwamish is generally between 2 and 3 meters, which is not unusual for an estuary.

Hull (1963, p. 604) found that the compensation depth in Baltimore Harbor ranged between 0.6 and 2.4 meters, with a mean of 1.7 meters. The importance of hydrographic factors in controlling bloom timing is shown by a significant correlation of productivity at station 7.7 during 1965–66 with measurements of relative stability (Δ°C) and retention time (fresh-water discharge).

The increase in temperature of the surface water probably has a greater effect on the timing of blooms by indirectly increasing stability in the photic zone than by directly influencing the photosynthetic rate. The observed decrease in daily maximum surface temperature of 4°C in 4 days at station 7.7 during the 1966 bloom (fig. 4) apparently did not directly influence the photosynthetic rate. On days when the daily maximum temperature was lowest, the daily maximum percent saturation of oxygen was greatest; this relationship indicates that photosynthetic oxygen evolution is not related to a temperature change of this magnitude. An increase in the vertical temperature gradient in the surface water would indirectly increase the stratification and also would reflect the extent of turbulent mixing. A vertical gradient in specific conductance might not always indicate stability when water from the salt wedge is continually added to the surface water of an estuary by strong tidal action and turbulent mixing. Under such conditions, an instantaneous specific-conductance profile might show a gradient, even though in reality a considerable exchange was occurring which would reduce the temperature gradient. During periods of less severe tidal action and turbulent mixing, the temperature gradient would tend to increase as the surface water was retained under high light intensity. Although correlation of the bloom periods with surface-to-bottom differences in temperature probably does not indicate a direct effect of temperature on photosynthetic rate, it does show the influence of surface-water stability on bloom development.

The conditions which permit bloom development are evidently too critical to be predicted solely by tidal-prism thickness and discharge. Favorable tidal-prism thicknesses and fresh-water discharges apparently occurred also during September of 1965 and 1966, but a second bloom did not develop in either year. However, light intensity and temperature in the surface water during these September periods were much lower than on about each August 1, preceding the bloom. Thus, while conditions of tidal action and discharge in these Septembers were favorable, the combination of reduced light and temperature may have resulted in insufficient stability, as well as insufficient light intensity, for the production of a bloom.

To facilitate prediction of the timing of blooms in the Duwamish, more frequent and specific measurements of stability related to pro-

ductivity should be made, particularly during the summer months, to provide a closer correlation. Vertical profiles of water velocity recorded at peaks of ebb and flood tides would probably correlate well with productivity because such measurements would tend to integrate the factors of vertical turbulence and retention time.

Hydrographic conditions during the summer of 1964 probably best explain why a phytoplankton bloom failed to develop then. The minimum fresh-water discharge of 760 m^3/min in August 1964, compared to about 550 m^3/min during the blooms in 1965 and 1966, was the highest minimum flow of record since the start of data collections in 1937. The greater minimum flow in 1964 probably created conditions of greater turbulent mixing in the photic zone and of shorter retention times; both conditions could have prevented bloom development. Also the mean temperatures in the surface water were lower during the summer of 1964 than in 1965 and 1966. Thus, available light, turbulent mixing, and retention time probably were not as favorable to phytoplankton growth during 1964 as they were during 1965 and 1966.

The importance of stability and compensation depth in controlling the timing of phytoplankton blooms is well known in nearshore environments. Riley (1942, p. 78) found that although nutrient concentrations are abundant in north-temperate waters during the winter, the spring phytoplankton blooms do not occur until March or April, when stability increases. The rate of increase in the phytoplankton population on Georges Bank, Mass., varied directly with the reciprocal of the depth of vertical-turbulence when the photic-zone thickness and the rates of photosynthesis and respiration were constant. Stability was also important in promoting bloom conditions in Block Island Sound, off the Connecticut-Rhode Island coast (Riley, 1952). Gran and Braarud (1935) found that stability controlled the timing of blooms in the nutrient-rich waters of the Bay of Fundy. Although nutrient concentrations were always high, strong vertical turbulence did not allow the phytoplankton cells to remain in the illuminated zone long enough to utilize the nutrients. A bloom resulted when the zone of vertical turbulence was shallower than the photic zone, a condition that permitted production to exceed respiration. Smayda (1957, p. 355) pointed out that although the maximum winter phytoplankton population in Narragansett Bay occurs during a period of unstable conditions, the shallowness and weak currents characteristic of the water body are sufficiently confining for population development. Gilmartin (1964, p. 530) found a close correspondence between phytoplankton production and vertical stability in a British Columbia fjord.

Although the timing of the spring bloom in north-temperate oceanic waters is determined primarily by light availability, compensation depth, and depth of vertical turbulence, the magnitude of the bloom is generally limited by nutrient supply (Anderson and Banse, 1961, p. 62–63). Pratt (1965, p. 182) concluded that the maximum biomass attained during the diatom flowering in Narragansett Bay was controlled by the nitrate and silicate content. Growth was observed to continue after exhaustion of the nitrate during most years, but never after depletion of the silicate. The foregoing observations help to establish that the increased nutrients in the Duwamish estuary probably would tend to increase the maximum biomass attained during a bloom rather than to alter its timing.[1]

EFFECT OF NUTRIENTS ON MAXIMUM BIOMASS PRODUCED

Limited experimental evidence indicates that effluents added to Duwamish River water stimulate the rate of nutrient assimilation by green algae. This suggests that the biomass attained in a bloom or in the total annual production might increase in response to increased nutrients from the RTP; however, field data during 1965–66 indicate that this did not occur. The annual mean biomass (chlorophyll *a* concentration) in 1966 was not significantly greater than the mean biomass in 1965 even though the mean effluent discharge was 46 percent greater in 1966. Nevertheless, this conclusion is only tentative because sampling frequency during the bloom may not have been adequate to define the total biomass produced.

Results of the in vitro bioassay cannot be automatically interpreted as evidence that added nutrients in the effluent will increase the bloom biomass because the response measured was from green algae, not from the diatoms that were present in the blooms. Further, the experiments were performed in the spring rather than during the bloom period; as Fournier (1966, p. 18) pointed out, the physiological response of phythoplankton communities to nutrient enrichment varies with the season and the physiological state of the community (that is, its position on the theoretical growth curve).

Examples of phytoplankton production that increased as a result of nutrient increments from domestic wastes are largely for water bodies in which preeffluent nutrient concentrations were much lower than those in the Duwamish. Edmondson, Anderson, and Peterson (1956, p. 50) reported maximum phosphate concentrations of 0.022, 0.020, and 0.038 mg P/l in the hypolimnion of Lake Washington during the summers of 1933, 1950, and 1955, respectively. The minimum mean

[1] Silicate is not one of the limiting nutrients in the Duwamish estuary because it is always an abundant constituent of the natural fresh-water inflow.

value for the hypolimnion in 1933 was about 0.005 mg P/l. The increase in phosphate during 1950–55 was attributed to an increased inflow of treated domestic waste and was accompanied by greater productivity in the lake. Sawyer (1952) concluded from a study of several Wisconsin lakes that an inorganic phosphate concentration of 0.01 mg/l was sufficient to promote bloom conditions. Goldman and Carter (1965, p. 1058) found in bioassay studies that phytoplankton in the highly oligotrophic Lake Tahoe responded to very small additions of treated domestic waste effluent. The maximum concentrations of phosphate and nitrate in the lake were very low (0.007 mg P/1 and 0.015 mg N/1, respectively).

Preeffluent nutrient concentrations in the Duwamish were much greater than those that reportedly promote accelerated eutrophication in lakes. A decline in nutrient concentrations to limiting levels was not apparent during the blooms; however, additional effluent nutrients in excess of the present (1966) relatively large quantities might increase the bloom biomass. Ryther, Yentsch, Hulbert, and Vaccaro (1958) studied a phytoplankton bloom in a small tidal creek tributary to Long Island Sound. They found that a large population, in which chlorophyll *a* concentration ranged from 116 to 245 mg/m^3, seemed to be limited in growth by ambient nitrate concentrations of 0.05 mg N/l and phosphate concentrations of 0.12 mg P/l. The possibility that these nutrient concentrations limited growth was indicated by the comparison of the high photosynthetic rate of the population (5 g C/m^2/day) with the rate of phosphorus and nitrogen uptake that would be necessary for growth to occur; the ratio of 100 : 15 : 1 for C : N : P was used. The necessary daily supply exceeded the amounts of the two nutrients available. Shapiro and Ribeiro (1965) found that although phosphate was relatively high in the Potomac River (0.007–0.15 mg P/l) upstream from an outfall for domestic waste, effluent phosphate added to river water that initially contained 0.037 mg P/l increased the in vitro growth of green and blue-green algae. The increase in growth was proportional to the added concentrations of phosphate, which ranged from 0.295 to 2.10 mg P/l. These results from the Potomac were generally similar to those found in the in vitro bioassay studies with Duwamish water; however, extrapolation of experimental data of this type to predict field conditions would have only limited value at best. As pointed out by Talling (1962, p. 747), algae grown in cultures often require much greater concentrations of nitrogen and phosphorus than algae growing under field conditions partly because cultures have more rapid growth rates than natural-growing algae and higher cell densities. Thus, ambient concentrations of phosphate which are not limiting to algae growing in an estuary may well become

limiting to algae growing in water confined within a flask. To predict more reliably the effect of future increases in RTP effluent on total phytoplankton production in the Duwamish estuary, in situ nutrient addition experiments are recommended. These should be made in large polyethylene test cells during periods of active growth, according to procedures outlined by Goldman and Carter (1965, p. 1048).

RELATION OF PHYTOPLANKTON TO DISSOLVED OXYGEN

If total phytoplankton production in the Duwamish increases as a result of nutrients added by the RTP effluent, the late summer depression of DO will probably increase in extent and persistence as a result of the added BOD from the phytoplankton. Minimum DO occurs in the upstream end of the saline-water wedge presumably because increased retention of this unaerated water mass during periods of minimum discharge and tidal-prism thickness allows more time for the existing BOD to act. Water with maximum BOD near stream bottom was observed consistently at station 9.7 regardless of tide stage even though the water with minimum DO occurred downstream from that point on low tide and upstream on high tide. This implies a mechanism by which oxidizable particulate matter is continually being resuspended and redeposited in this transition area as the bottom water moves upstream and downstream with the high and low tide. The BOD has ample time to act during periods of prolonged retention; the bottom water probably traverses the area many times without reaeration before it is finally removed from the estuary by entrainment in the surface water.

A large amount of the BOD in this bottom water originated from phytoplankton that sank from the near-surface waters. This fact was shown by a significant correlation between BOD and phytoplankton biomass (chlorophyll *a* concentration) in all low-tide samples collected throughout the summer of 1966, as well as by close correlations of these two variables longitudinally in the estuary and seasonally at stations in the transition area where the maximum values were observed.

Because the regression line for chlorophyll *a* and BOD intercepts the BOD ordinate (fig. 22), some of the BOD probably was not due to chlorophyll *a*. Undoubtedly organic particulate matter from sources other than phytoplankton settles in the transition area and contributes to the BOD. A BOD, however, can be exerted by phytoplankton organic matter lacking chlorophyll *a*, as was pointed out earlier. Organic detritus from upstream and solids from the numerous but relatively small organic waste effluents in the area are probable sources of BOD. The amount of BOD contributed from various sources is

unknown, however, and the organic carbon produced by phytoplankton alone represents a BOD source capable of extensive DO depletion. During the bloom in 1966, the mean of the gross productivity rate measured by the oxygen method and by the carbon-14 method was about 3.0 g/C/m^2/day. The conditions of chlorophyll *a* concentrations greater than 50 mg/m^3 in the bottom water and cell concentrations there of about 11,000 per milliliter when chlorophyll *a* is comparatively low (5.5 mg/m^3) indicate that a large proportion of cells produced in the surface water sink into the bottom-water layer. Even if phytoplankton sink in the reach, toward the bay, some could ultimately be deposited in the transition area because the net flow of the bottom water is upstream (J. D. Stoner, U.S. Geol. Survey, written commun., 1967). Another indication that most of the phytoplankton organic matter is deposited in the upper transition area is the comparatively low concentration of phytoplankton in the lower estuary (station 1.9), however, sea-water dilution may have reduced the concentration by as much as 50 percent. About two-thirds of the organic matter produced in the surface layers (or about 2 mg C/m^2/day) probably sinks into the bottom-water layer. Twenty days (the approximate duration of the bloom in 1966) of production at this rate would result in an equivalent oxygen consumption of 120 gm O_2/m^2 (for 5 days, the value would be 30 g O_2/m^2). The saline-water wedge has an estimated mean depth of about 5 m at station 7.7 for a complete tidal cycle, and the mean depth undoubtedly is much less at station 9.7. Assuming an even distribution of organic matter in a 5-m column, the 20-day BOD would be 24 mg/l (24 g O_2/m^3), and the 5-day BOD would be 6 mg/l. The distribution would not be uniform, however, because the particulate matter, and hence the BOD, would probably be concentrated near the bottom. The farthest upstream segment of the saline-water wedge would probably be the oldest water in the estuary, and its volume, upon which the particulate BOD could act, would be the smallest. Thus, the low DO at low tide can be explained by the respiration and decomposition of large phytoplankton concentrations in a rather small isolated volume of water in the estuary.

The effect of large concentrations of decomposing algae on DO has been shown by workers in other areas. Sinking organic matter produced by phytoplankton blooms results in a hypolimnetic oxygen deficit in lakes; this deficit in turn is a measure of the productivity and the degree of eutrophication (Edmondson and others, 1956). Barlow, Lorenzen, and Myren (1963, p. 251, 261) observed similar conditions in the Forge River, Long Island estuary. There, planktonic organic matter that was produced locally because of relatively great nutrient supplies exceeded the organic matter inflow from other sources, thereby resulting in anerobic conditions in the bottom mud below the photic

zone. In other fresh-water environments, Bartsch (1960) has shown that the BOD from algae produced as a result of added effluent nutrients has exceeded the demand of the original waste. Nash (1947, p. 160) measured minimum DO in the bottom water of the Patuxent River estuary, Maryland, during maximum stratification in May and June. (Although he suggests that bacterial respiration and increased stratification of the bottom layer caused the DO to decrease, he did not mention the potential BOD in the spring phytoplankton bloom, which occurred in April and May preceding the oxygen decrease.)

Further depression of DO minimums in the Duwamish estuary seems likely if a substantial increase in phytoplankton production results from the anticipated increase in effluent nutrients. Although the timing of phytoplankton blooms seems to be strongly controlled by hydrographic factors, nutrient increases may in turn increase the maximum biomass produced in the bloom. This condition has not happened yet; moreover a comparison of the mean annual biomass of 1965 to that of 1966 shows no significant difference despite a 46-percent increase in effluent discharge. However, the conclusion that nutrient increases may effectively increase biomass production is only tentative, because the number of individual measurements of maximum productivity and chlorophyll *a* was much greater in 1966 than in 1965. In order to define the total biomass produced, increased sampling frequency is certainly warranted during the bloom periods. The increase in effluent discharge, if the discharge is cool enough to mix well with the river water, may also have an inhibiting effect on phytoplankton productivity by increasing turbulence in the photic zone. Such an effect would probably require a substantial increase in effluent discharge, which is not expected in the near future.

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