

AN ABSTRACT OF THE THESIS OF

HARVEY DEAN WILLIAMS for the Ph. D.
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✓ Dr. Harry K. Phinney

Raw and biologically stabilized Kraft mill effluents were introduced into laboratory artificial streams at a ratio of 15 ml per liter of water over a two year period. Effluent from the stabilization pond of a second mill was introduced at concentrations of from 5 to 40 ml per liter of water for a period of three months. Trays bearing portions of intact periphyton communities growing on rock rubble were removed from the streams and placed in a photosynthesis-respiration chamber where rates of oxygen production and consumption were measured. Comparisons were made between experimental and control streams on the basis of rates of oxygen production and consumption, P/R ratios, organic matter per unit area, concentration of chlorophyll a, mg of oxygen produced per hour per mg of chlorophyll a, relative frequency of occurrence of filamentous algae and densities of populations of diatoms.

Communities from streams receiving raw effluent tended to

be more heterotrophic than control communities while communities from streams receiving stabilized effluent tended to be more autotrophic than controls. Stabilized effluent produced a greater number of significant differences in community function and structure than did raw effluent. Nitrates and phosphates added in the stabilization process may have contributed to the greater observed effect.

The diatoms Navicula sp., Cocconeis placentula var. euglypta, and Synedra ulna were significantly more abundant in streams receiving raw effluent than in controls. Rhoicosphenia curvata, Fragilaria brevistriata, C. placentula and Achnanthes minutissima were significantly less abundant.

Melosira varians, R. curvata and C. placentula were significantly more abundant and S. ulna was less abundant in streams receiving stabilized effluent from mill A than in controls. R. curvata increased in abundance with increasing concentrations of effluent from mill B. M. varians, F. brevistriata and S. ulna decreased with increasing concentrations of effluent from mill B. Total diatom population was significantly reduced by raw and stabilized effluent.

Oedogonium sp. was more abundant in streams receiving stabilized effluent. Organic matter and chlorophyll a per unit area decreased with increasing concentrations of effluent from mill B.

Effect of Kraft Mill Effluent on Structure
and Function of Periphyton Communities

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Redacted for Privacy

Professor of Botany
in charge of major

Redacted for Privacy

Chairman of Department of Botany

Redacted for Privacy

Dean of Graduate School

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EFFECT OF KRAFT MILL EFFLUENT ON STRUCTURE AND FUNCTION OF PERIPHYTON COMMUNITIES

INTRODUCTION

Throughout our industrial history, a common "trick of nature" has been the placing together of resources, the exploitation of which leads to conflict between the industries using them. Such has been the case with the wood pulp and fishing industries which in forested coastal areas often find themselves in conflict.

The contention of fisheries partisans that effluent from Kraft process pulp mills is harmful to fish and other aquatic life has been supported by a number of toxicity studies dating back to 1930 (Cole, 1935; Fujiya, 1961; Haydu, Amberg, and Dimick, 1952; Howard and Walden, 1965). This has led to efforts to identify and characterize toxic components of Kraft effluent by Webb (1958), Wiman (1962) and Howard (1965).

Of more recent concern, however, has been the effects on fish production by Kraft waste at a level below that causing acute toxicity. Research during the past three years at the Pacific Cooperative Water Pollution and Fish and Wildlife Laboratories and elsewhere has centered around these apparent effects (Servizi, Stone and Gordon, 1966; Ellis, 1968; Tokar and Owens, 1968; Sprague and McLeese, 1969). It has not been determined whether the effects of

low concentrations of effluent likely to be discharged into streams, are due to the action of effluent directly upon fish, inhibiting growth in some as yet obscure manner or indirectly through the aquatic food chain upon which fish subsist.

In the lotic environment, the periphyton or aufwuchs together with terrestrial debris comprise the base of the food chain. Herbivorous insect larvae feed directly upon the periphyton and whatever edible debris happens to fall into the stream. Interpreted in terms of Lindeman's trophic dynamics scheme (1942) energy stored by these organisms is passed up the food chain through predation to higher trophic levels.

Attempts have been made to identify effects of Kraft mill effluent on food chain invertebrates by Dewitt (1949) and Van Horn Anderson, and Katz (1949). However, literature reviews and a personal search of the literature revealed no published studies dealing with the effects of Kraft wastes on lotic primary producers (Eldridge, 1963; McFarlane, 1963; Gellman, 1966; Gehm and Gove, 1968; Gove, 1968). Indeed only a single study of the effects on freshwater primary producers has appeared and that dealt with plankton algae in a stabilization pond of a paper mill (Oliver and Dorris, 1964).

By considering both aspects of the study, that dealing with the effects of Kraft effluent on production by salmonid fish by a fisheries

investigator and that relating to the structure and function of periphyton communities, the subject of this thesis, in the simplified ecosystem provided by laboratory streams, it was possible to obtain a more complete and unified picture of the total effects of mill effluent on the entire food chain. Although natural streams receiving effluent would have provided more realistic models for study, the laboratory streams allowed better controls and replication of experiments. Such streams have provided valuable information regarding the structure and function of periphyton communities in earlier studies (Odum and Hoskins, 1957; Beyers, 1963; Davis, 1963; McIntire et al., 1964; Kevern, 1965; McIntire and Phinney, 1965; McIntire, 1966a, 1966b, 1968c).

A stream unaffected by waste products of human origin exists in a steady state supporting a flora and fauna uniquely adapted to the environment. Margaleff (1960) has observed that the uniqueness of each stream is enhanced by the area it drains. Butcher (1947) expresses the same principle another way when he notes that eutrophication is a natural process occurring as a river flows toward the sea.

When man disturbs the drainage area of the stream or utilizes the stream as a conduit for removal of waste products such as Kraft mill effluent, the process of eutrophication may be accelerated resulting in modification of environmental conditions in the

stream. When the quality of the water in a stream is changed to such an extent that man finds it offensive to him or unusable for some purpose, he is prone to describe the stream as being polluted, a term too lacking in qualitative or quantitative expression for meaningful communication. A truly adequate description of stream conditions demands a more objective basis.

Fjerdingstad (1950) suggests three possible methods of measuring and expressing water quality: physico-chemical, in which individual factors such as dissolved oxygen or water temperatures are recorded with no regard to possible synergistic effects on organisms; bacteriological, concerned mainly with fecal contamination; and biological involving quantitative observations on populations inhabiting the water. Since it is the effects of environmental conditions on some population or assemblage of populations that is most apt to be of concern and since populations of organisms will reflect the presence of synergistic effects, the biological approach would seem to be preferable.

Biological criteria of water quality have been applied for 50 years but more emphasis has been placed recently on primary producers as indices of quality as a result of the work of Patrick (1949, 1950, 1963); Patrick, Hohn, and Wallace (1954); Patrick and Strawbridge (1963); and Beak (1964), Patrick and her co-workers having refined the concepts of species diversity and indicator

associations of diatom species as means of estimating the effects of pollution.

The advantages notwithstanding, application of biological methods of evaluating water quality based on primary producers presents special problems in flowing waters. Because the primary producers in a lotic environment are attached to heterogeneous substrates, obtaining representative samples of periphyton communities is difficult. The problem is further complicated by the effects of current velocity first noted by Fritsch (1929) when he observed the unique nature of the encrusting algal flora typical of certain fast flowing streams. This was noted later also by Butcher (1946) and most recently studied by McIntire (1966b) who attempted to quantify the effect of current.

However, the homogeneous nature of the substrate material used in laboratory streams and the ease with which current velocity can be held constant obviates these problems.

METHODS AND MATERIALS

Kraft Mill Effluent

The three types of Kraft mill effluent studied were:

1. Effluent taken from the settling basin of a Kraft pulp mill and introduced into the streams without prior treatment. To prevent microbial action, this effluent was stored in refrigerated tanks until used.
2. Effluent taken from the same settling basin and stabilized by the action of sewage bacteria during storage before introduction into the experimental streams.
3. Effluent taken from a stabilization lagoon of a second mill and introduced into the streams without further treatment.

The mill from which material for treatments number one and two above was obtained will be designated mill A and that from which the waste described in treatment number three was obtained will be designated mill B.

Effluent from mill A was collected weekly from the outfall of the settling lagoon of a Kraft mill producing unbleached pulp. The liquid was hauled to the laboratory in 50-gallon drums lined with polyethylene. When the raw waste was to be used, the storage tanks

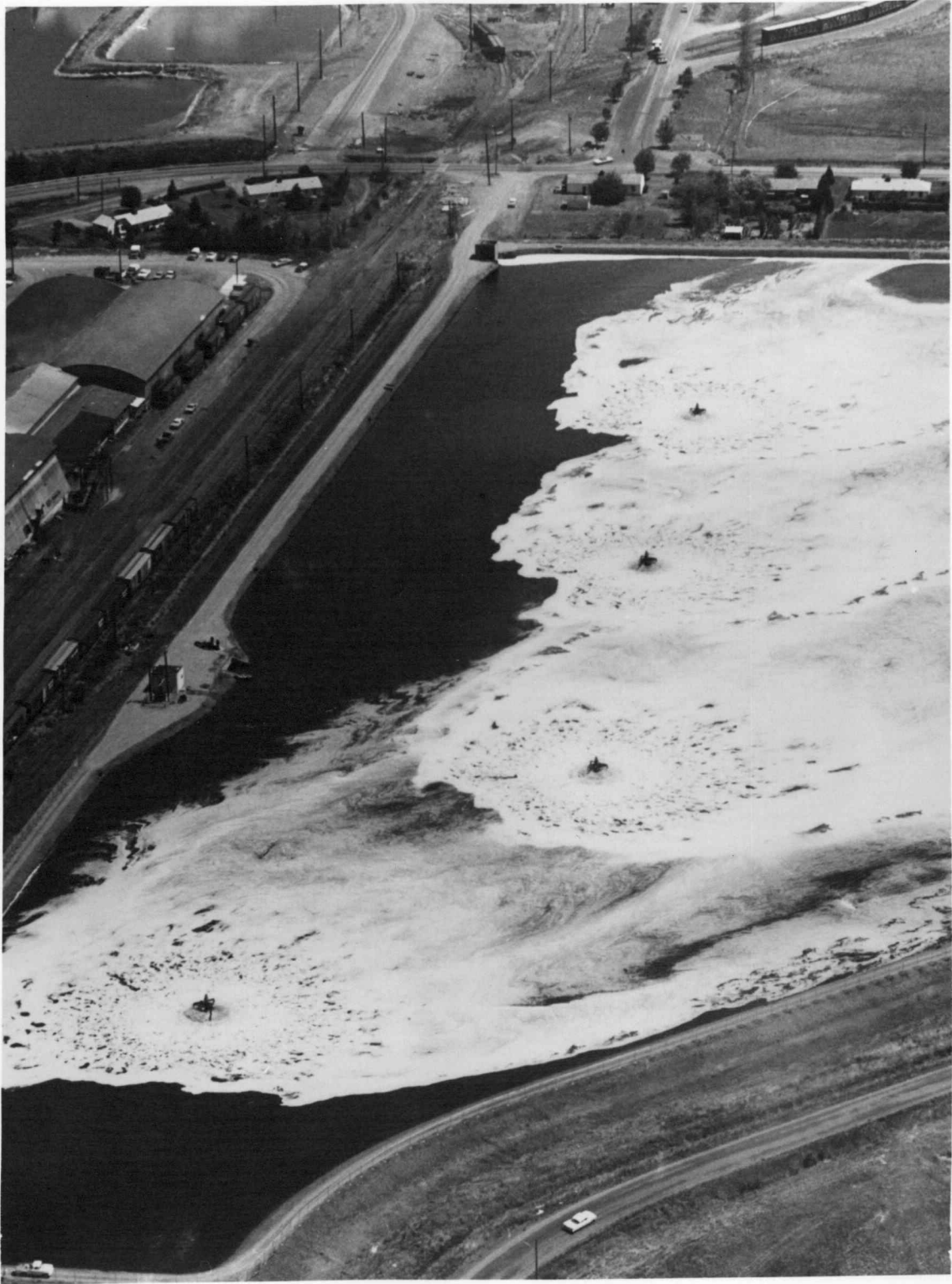


PLATE I Four 75 horsepower aerators floating in the stabilization pond at mill B.

were refrigerated. Treated waste was stored unrefrigerated.

Stabilization of the waste in the laboratory was accomplished by action of sewage bacteria reducing the biochemical oxygen demand (BOD). After collection, the waste from mill A was placed in an 800 gallon redwood tank, nitrogen in the form of NH_4NO_3 or KNO_3 and phosphorus in the form of monobasic phosphates were added on the basis of one ppm elementary nitrogen to 10 ppm BOD and one ppm elementary phosphorus for 50 ppm BOD, followed by aeration for one week.

The initial batch was seeded with sewage sludge but seeding of subsequent batches relied upon residual bacteria from the previous batch. The stabilization process reduced the 5-day BOD (BOD_5) from an average of 250 ppm to between six and eight ppm although on one occasion, a post-stabilization BOD_5 of 72 ppm was recorded.

Stabilization of the Kraft waste from mill B took place in a large pond, 21 acres in area, 10 to 12 feet deep with a capacity of 80,000,000 gallons. Aeration was by five 75 horsepower Yeoman Brothers aerators. No nutrients were added since sufficient nitrogen and phosphorus were already present in the effluent. The retention time of eight days was sufficient to lower the average BOD_5 from 200 ppm to 16 ppm (Bishop et al., 1968).

Samples of each batch of waste were analyzed by the National Council for Air and Stream Improvement laboratory at Oregon State



PLATE II 75 horsepower floating aerator churning air into Kraft mill effluent pond. Mixing is by a large propeller-like beater.

University where BOD_5 , volatile solids, total dissolved solids and chemical oxygen demand (COD) were determined. These data are summarized in Appendix Tables 12, 13, and 14.

The Kraft or sulfite pulping process utilizes an alkaline liquor containing sodium hydroxide and sodium sulfide to cause separation of cellulose fibers from lignin and other components of the cell wall. Wood chips are cooked in the alkaline liquor until digestion occurs resulting in a solid residue of cellulose fibers and a liquid fraction that because of its dark color is referred to as "black liquor."

The cellulose fibers are washed and manufactured into paper and paper products while the black liquor is concentrated in vacuo and burned or sold for further refining. The mill effluent results primarily from the washing process which requires 20 to 30,000 gallons of water per ton of wood pulp. Hence, an average size mill producing 500 tons per day of wood pulp will discharge 25,000,000 gallons of wash water (Berger, 1966).

The composition of Kraft mill effluent varies with location depending upon the species being pulped, the temperature and duration of cooking and even the time the wood chips are stored prior to pulping. Typically, the BOD_5 of Kraft mill effluents ranges around 250 mg of oxygen per liter; total solids, 1700-2500 mg per liter, 65% of which are volatile; alkalinity from 100-300 mg per liter; and pH from 7.5 to 9.0 (Wiman, 1962).

Although the biochemical oxygen demand of Kraft mill waste is low by comparison with other waste materials discharged into natural waters, the great volume of discharge from a single mill makes it a problem.

Wiman (1962) analyzed Kraft mill waste using material taken from the low vacuum stage of the evaporators and found benzene, acetone, ethers and two groups of constituents which he divided into the major and minor products. The major product he found to be a para substituted aromatic compound, 4-(p-tolyl)-1-pentanol, a substance found to equal cyanide in its ability to inhibit the cytochrome oxidase enzyme system in vitro. The minor products were identified as terpenes and included limonene, gamma-terpinene, p-cymene and anisol.

Laboratory Streams

The laboratory streams were housed in a building with a roof of translucent fiberglass sloped toward the south. To insure adequate ventilation and to prevent excessive heating due to the greenhouse effect, the north and south sides of the building were enclosed only to height of five feet leaving an open space of three and five feet, respectively, on the two sides.

Although the translucent roof absorbed 60% of the incident light, excessive growth of filamentous algae in the streams seemed

to inhibit the growth of the fish making necessary further reduction of light intensity. This was accomplished by suspending saran shading cloth at a height of 4' above the streams reducing the intensity of light reaching the streams to 8% of the incident light.

The streams consisted of six wooden troughs 66 cm in width, 25 cm in depth, and 3.3 meters in length. Each stream was separated into two parts by a longitudinal partition. Openings at each end of the partitions allowed water to circulate on both sides of the partition. All wooden surfaces were sealed with a non-toxic white paint.

The streams were divided into riffle and pool areas by the addition of styrofoam sheets elevating a section of bottom on each side of the partition. The ends of the streams then became pools and the elevated areas produced riffles.

The purpose of the riffle-pool arrangement was to provide hiding places for the young salmon and by varying the environment to simulate more closely natural stream conditions. Of the total area of 2.10 square meters, 1.55 square meters was riffle area and 0.55 square meters was pool.

The stream bottoms were covered with smooth river rubble. Care was taken to insure that each stream received the same volume of rubble similar in size to that of every other stream.

A current of 21.9 cm sec^{-1} was produced by aluminum paddle wheels driven by constant speed motors. Filtered spring water was

circulated through the streams at the rate of two liters per minute exiting by means of stand pipes that maintained constant levels of water in the streams. The total volume of water contained in each stream was 215 liters.

Pulp waste was introduced to the streams through neoprene tubing by a peristaltic pump. Daily adjustments of the flow of both water and pulp waste assured maintenance of uniform concentrations of pulp effluent in the streams.

To allow the removal of intact portions of the periphyton communities, two trays made of marine grade plywood were placed beneath the rubble in the riffle area, one on each side of the partition. Water temperature was monitored continuously by means of a Marshalltown model 1000 thermograph. Weekly minimum and maximum temperatures are presented in Appendix Figure 1.

The quality of the water drawn from the springs used as a water source in these experiments has been relatively consistent over the years it has been used. Records of past analyses indicate a pattern of gradual increase in total dissolved solids, specific conductance, pH, and alkalinity during the spring and summer followed by a decrease in the fall and winter. These data are summarized for the years 1961-63 in Appendix Table 15.

The communities in the streams were seeded naturally by organisms entering with the water supply. Seeding also occurred

when bottom detritus from a nearby stream was added when the streams were stocked with insect larvae. Some seeding may have taken place with the introduction of the effluent, but examination of centrifuged samples of effluent indicated that it did not occur.

Raw and stabilized effluent was introduced into experimental streams according to the following schedule:

Experimental treatment	from	to	ml effluent/ml water
raw effluent (mill A)	7/66	7/67	15
stabilized effluent			
mill A	7/67	5/68	15
	5/68	7/68	40
mill B	7/6	10/68	5, 10, 20, 40

Sampling periods were dictated by the duration and timing of the study of fish production. Material could not be removed from the streams while these experiments lasting from six to eight weeks each were in progress. Sampling was especially difficult during the first year because of problems experienced in getting the fish to grow in the streams. Of great concern was the lush growth of filamentous algae during the summer of 1966 that was suspected of interfering with the ability of the fish to feed.

In September, all the bottom rubble in the streams was removed and scrubbed clean in an effort to reduce the amount of algae. The attempt failed and the algae began to regenerate to its former

level. In a further effort to control algal growth, the streams were planted with large numbers of the snail Oxytrema silicula. This attempt succeeded beyond expectation when the snails grazed the periphyton nearly to extinction. The snails were removed on November 19 but since day length had shortened and the streams were receiving less solar energy, regeneration of the periphyton communities was slow.

By March, excessive growth of algae had become a problem again so saran greenhouse shading screen was installed over the streams. During the four-month period of colonization subsequent to scrubbing in September, the streams were stocked six times with aquatic insect larvae collected from Berry Creek, a small woodland stream 12 miles north of Corvallis. A later stocking occurred on April 7, 1967.

Photosynthesis-Respiration Chamber

Rates of photosynthesis and respiration by intact periphyton communities were measured in a photosynthesis-respiration (P-R) chamber identical to that described by McIntire et al. (1964). The chamber consists of a rectangular porcelainized sheet steel tank covered by a transparent lucite lid sealed with a rubber gasket and held in place by C-clamps. The P-R chamber is 60 cm in length, 50 cm in width, and 17 cm in depth with flanges around the top for

clamping the lid. A pair of small centrifugal pumps circulated water through tubulations attached at appropriate positions to provide a current of water through the chamber.

Temperature in the P-R chamber was maintained at the level of the streams by circulating water supplying the laboratory streams through a water jacket. Illumination of 9,150 lux, less than the summer maximum of 11,100 lux, was provided by a 1500 watt incandescent lamp suspended over the chamber. The light from this source was diffused as it entered the chamber by a sheet of flashed opal glass placed over the lucite cover.

By closing the outlet of the chamber before the inlet was closed, pressure was built up inside causing the lucite top to bulge upward. This allowed the removal of up to two liters of water without replacement for dissolved oxygen measurements.

Samples of the periphyton communities used for measurements in the P-R chamber consisted of two trays bearing intact portions of the communities one from each of two streams receiving identical treatment. The measured values represented an average of the two trays. Sampling two trays at once was necessary to provide adequate samples for use in the P-R chamber. If both trays had been obtained from a single stream this would not have left sufficient material for my co-worker to sample the insect population.

When the trays were sealed in the chamber, water from the

supply to the laboratory streams was circulated through the chamber for six hours. At the end of this six-hour acclimation period, light was excluded and respiration measurements made.

Initially, respiration rates were measured at night so as to coincide with the natural diurnal cycle of the communities but since no difference was noted between night and day respiration rates, the more convenient hour was used.

In order that experimental and control communities could be exposed to light of equal intensity during photosynthesis measurements, an artificial light source was used. Because of the impracticability of excluding daylight, these measurements were made at night. Possible diurnal effects were minimized by turning on the artificial light before dark and in effect lengthening the daylight period.

Supersaturation with O_2 and the formation of O_2 bubbles in the chamber during photosynthesis were prevented by reducing the level of dissolved oxygen in the water supplying the P-R chamber to 7-8 mg per liter. This reduction was achieved by passing the water entering the chamber through a glass sparging column packed with raschig rings against a counter flow of nitrogen bubbles.

Measurements of photosynthesis and respiration were made for two successive one-hour periods then averaged to estimate the rate of photosynthesis or respiration. If the hourly rates varied by more

than 10%, the rate was measured for a third one-hour period and the average of all three used. The values obtained in successive hours proved to be remarkably consistent so that only occasionally was a third hourly measurement required.

Sampling Methods

Following measurement of respiration and photosynthesis, the periphyton was scrubbed from the rubble into four liters of water. The resulting suspension was homogenized for ten seconds in a large Waring blender.

The biomass (ash-free dry weight of organic material) was estimated by evaporating to dryness one liter of the resulting slurry and weighing the residue. A subsample of the dried material was combusted to determine the percentage of ash and this percentage of the dry weight deducted to determine ash-free dry weight i.e., organic matter.

The residue from a 15 ml sub-sample of slurry filtered through a HA 0.45 μm filter was placed in 25 ml of 90% acetone, 10% distilled water for determination of chlorophyll a by the method of Parsons and Strickland (1963). No attempt was made to assess the amount of degraded chlorophyll that might have been present since this has not been demonstrated to be important in flowing waters (Moss, 1967).

Duplicate 20 ml subsamples were taken, one for use in

preparation of diatom slides and the other preserved for later examination of the algae other than diatoms.

Diatom slides were prepared by oxidation of organic material with nitric acid and potassium dichromate in the conventional manner, dilution to standard volume followed by drying 3/19 ml of the suspension of diatom frustules on a cover slip and mounting in hyrax. Careful standardization of volumes allowed diatom counts on prepared slides to be related to population densities in the community.

Caloric content of the organic material was measured in a Parr combustion calorimeter number 1411, in accordance with Parr Instrument Company Instruction Manual No. 128 (1958).

Dissolved Oxygen Measurements

Measurements of dissolved oxygen during the winter and spring of 1967 were by the azide modification of the Winkler method (American Public Health Association, 1965). From the summer of 1967 on, dissolved oxygen was measured polarographically with a YSI oxygen meter model 54 and YSI model 5420 Clark type polarographic probe equipped with an agitator and fitted with a high sensitivity membrane. Prior to each use, the oxygen meter was standardized against the modified Winkler. The oxygen probe made unnecessary the taking of water samples from the P-R chamber. Instead, the probe was inserted directly into the chamber through a hole in the cover.

RESULTS

The results of the study are presented in two parts, the first comprising estimates of parameters of community function and the second comprising estimates of parameters of community structure.

Community Function

Measured values of community functional properties are plotted in Figures 1 through 11 for the period of introduction of effluent from mill A.

On May 15, 1968 the concentration of effluent in experimental streams was raised to 40 ml/liter of water. Data represented by the July, 1968 points on Figures 1-11 is based upon this concentration.

Gross Primary Production

Communities from streams receiving raw effluent achieved a maximum rate of gross primary production per unit area of $390 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ in September, 1966. The maximum rate of gross production estimated for control streams of $357 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ occurred also in September, 1966. The minimum rate of gross production for communities developed in the presence of raw waste was $76 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ and for control streams $203 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ both

recorded in January, 1967 (Figure 1). The mean rate of gross production for communities from streams receiving raw waste was $238 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$, lower than the $304 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ recorded for communities from control streams (Table 1).

Communities from streams receiving stabilized effluent reached their maximum rate of gross primary production of $438 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ in November while communities from their controls attained their maximum of $326 \text{ mg hr}^{-1} \text{ m}^{-2}$ during February (Figure 1). Minimum rates of gross production were attained by communities from both experimental and control streams in December, 1968, the minimum for communities from experimental streams of $179 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ exceeding that of the controls of $159 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$.

The maximum rate of gross primary production per unit mass of organic matter by communities from experimental streams while they received raw effluent was $2.55 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ in June, 1967 (Figure 3). During the same period, communities from control streams reached the maximum of $5.95 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$. Communities from experimental streams reached the minimum production rate of $1.49 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ in January, 1967 while the minimum rate for their controls of $2.68 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ occurred in March, 1967.

Communities from streams receiving stabilized effluent achieved the maximum rate of production of $15 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$

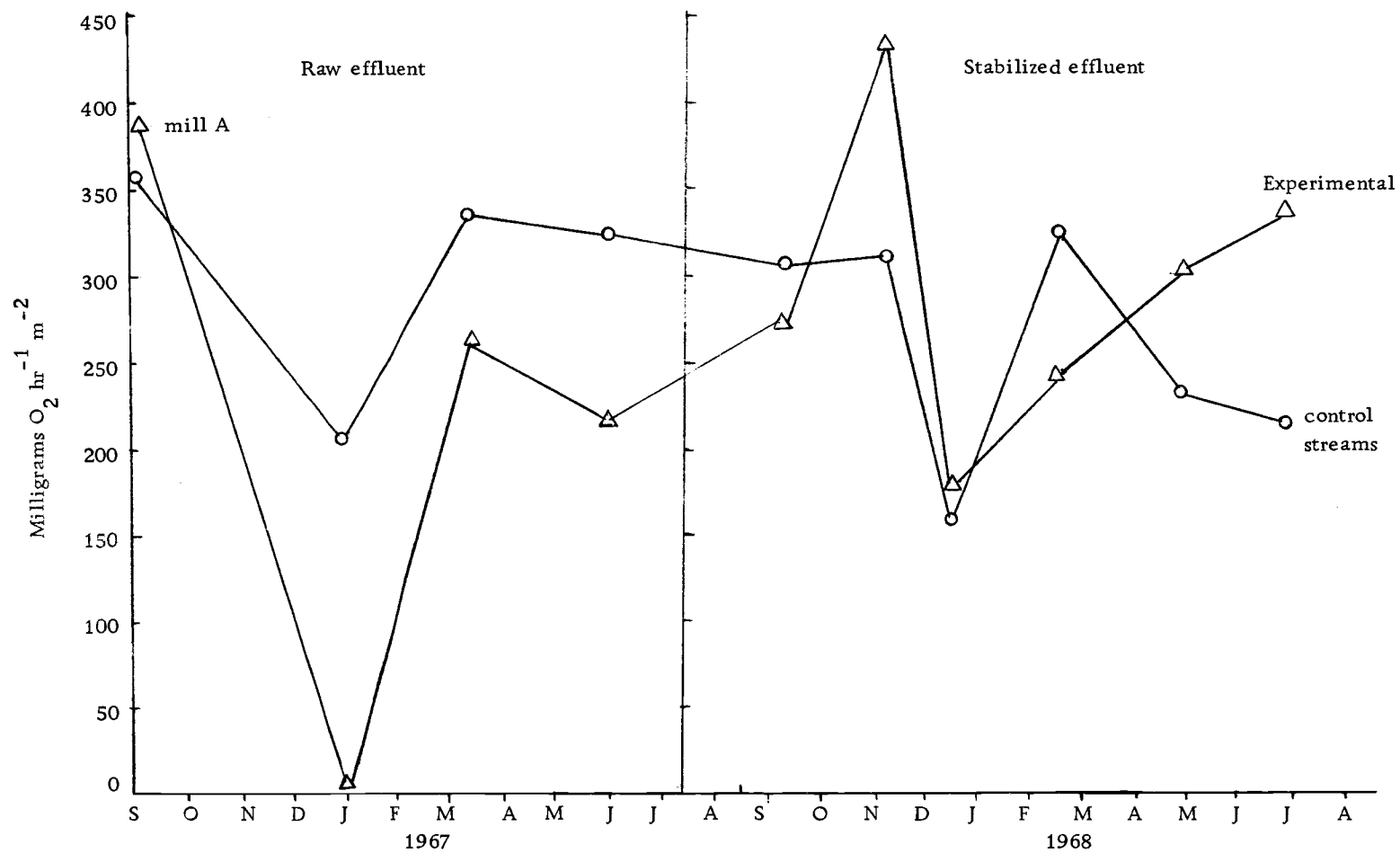


Figure 1. Rates of gross primary production as $\text{mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ as measured in the P-R chamber under 9, 150 lux illumination.

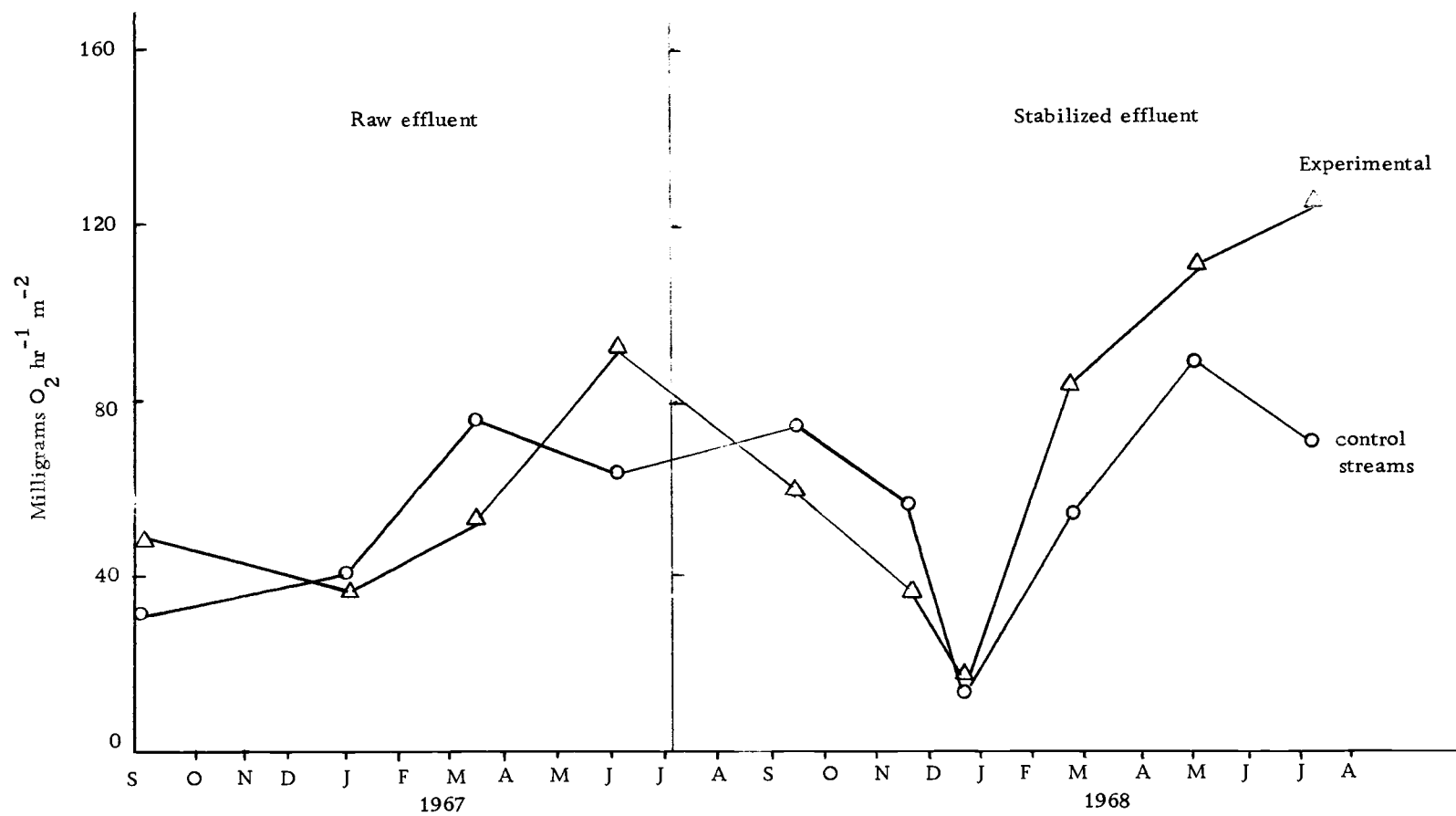


Figure 2. Rates of oxygen consumption as $mg\ O_2\ hr^{-1}\ m^{-2}$ as measured in the P-R chamber.

while their controls recorded their maximum rate of $11.2 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ of organic matter both in November, 1967 (Figure 2). The minimum rate of production of $5.10 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ was recorded for communities from experimental streams in September, 1967 and a minimum of $4.2 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ in controls during the month of February, 1967.

Mean gross primary production per unit mass of organic matter by communities from streams receiving raw effluent was $1.91 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ while controls averaged $4.81 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$.

Communities from streams receiving stabilized effluent produced an average of $9.82 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ compared to their controls which averaged $7.17 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$. Treatment with both raw and stabilized effluent produced statistically significant differences between mean production per unit mass of organic matter by communities from experimental and control streams (Table 1).

Oxygen Consumption in Respiration

Communities from experimental streams receiving raw effluent reached the maximum rate of oxygen consumption of $93 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ in June, 1967. Controls reached their maximum rate of oxygen consumption in March, 1967 when a rate of $76 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ was recorded. The minimum rate of oxygen consumption for communities from experimental streams of $37 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ was recorded in January 1967 while their controls reached a minimum

consumption of $30 \text{ mg hr}^{-1} \text{ m}^{-2}$ in September, 1966.

During the year when experimental streams were receiving stabilized effluent, their communities reached a maximum rate of oxygen consumption of $125 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ in July, 1968 while the controls registered a maximum rate of $90 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ in April, 1968. A minimum rate of oxygen consumption of $16 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ was reached by communities from experimental streams in December, 1967 and the controls, a minimum of 12 mg.

Mean rates of oxygen consumption by communities from experimental streams exceeded those of controls during both years of the experiment (Table 1). During the first year when experimental streams were receiving raw effluent, the mean rate of oxygen consumption of communities taken from them was $60 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ while the controls yielded a mean rate of oxygen consumption of $52 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$. The second year when stabilized effluent was being introduced into the streams, the mean rate of oxygen consumption by communities from experimental streams was $73 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$ and that of controls $60 \text{ mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$. The difference between rate of oxygen consumption by communities from experimental and control streams during introduction of raw effluent did not meet the 0.10 level of significance of Students' t-test while the difference between experimental and control communities during introduction of stabilized effluent did meet the 0.10 level of significance (Table 1).

Rates of oxygen consumption per gram of organic matter are presented in Figure 4. The maximum rate for communities from streams receiving raw effluent was $1.10 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ in June 1967 and controls, 1.17 in January 1967. The minimum rate for communities from streams receiving raw effluent recorded in March 1967 was $0.40 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ and for controls in the same month, $0.61 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$.

The maximum rate of oxygen consumption per unit mass of organic matter by communities from streams receiving stabilized effluent was $4.46 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ and $2.80 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ by controls, both in the month of July, 1968. The minimum rate recorded for communities from experimental streams was $0.47 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ and $0.53 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ for controls both in the month of December, 1967.

Oxygen consumption per gram of organic matter by communities from streams receiving raw effluent was exceeded by controls while communities from streams receiving stabilized effluent exceeded their controls in rate of oxygen consumption meeting the 0.10 Students' t-test criterion of significance (Table 1). Communities from streams receiving raw effluent averaged $0.66 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ and their controls $0.97 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$. Communities developed in streams receiving stabilized effluent averaged $2.33 \text{ mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ and control communities averaged $1.64 \text{ mg of O}_2 \text{ hr}^{-1} \text{ g}^{-1}$.

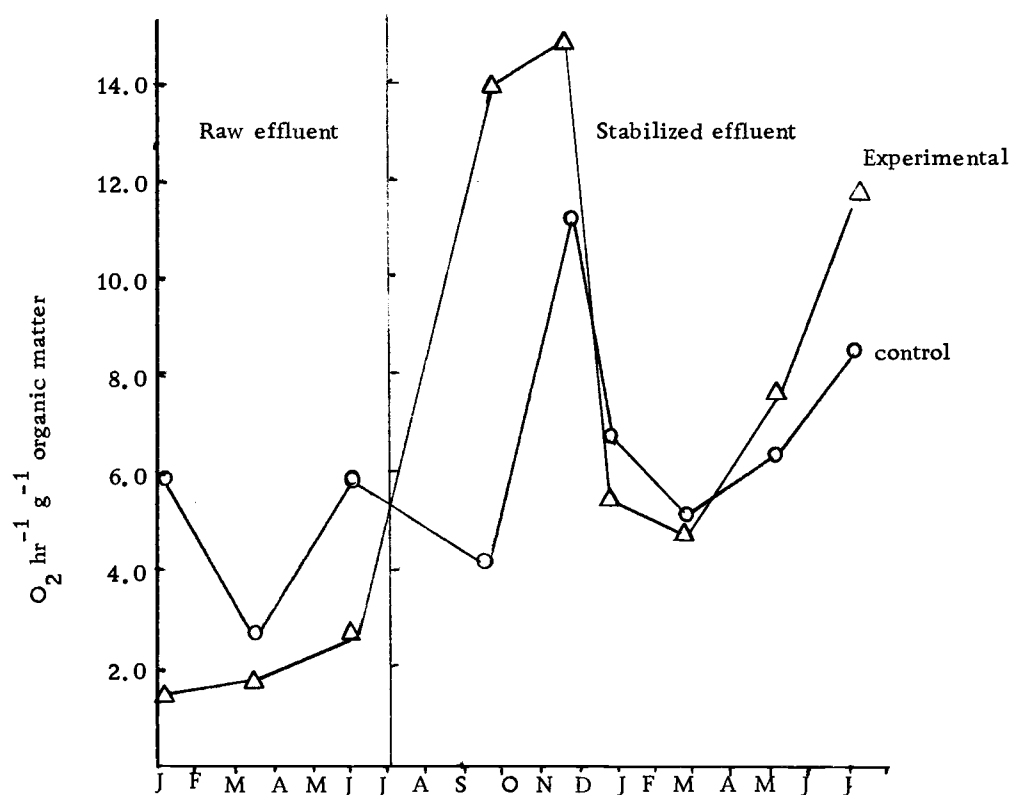


Figure 3. Rates of gross primary production as mg of O_2 hr^{-1} g^{-1} of organic matter as measured in the P-R chamber under 9,150 lux illumination.

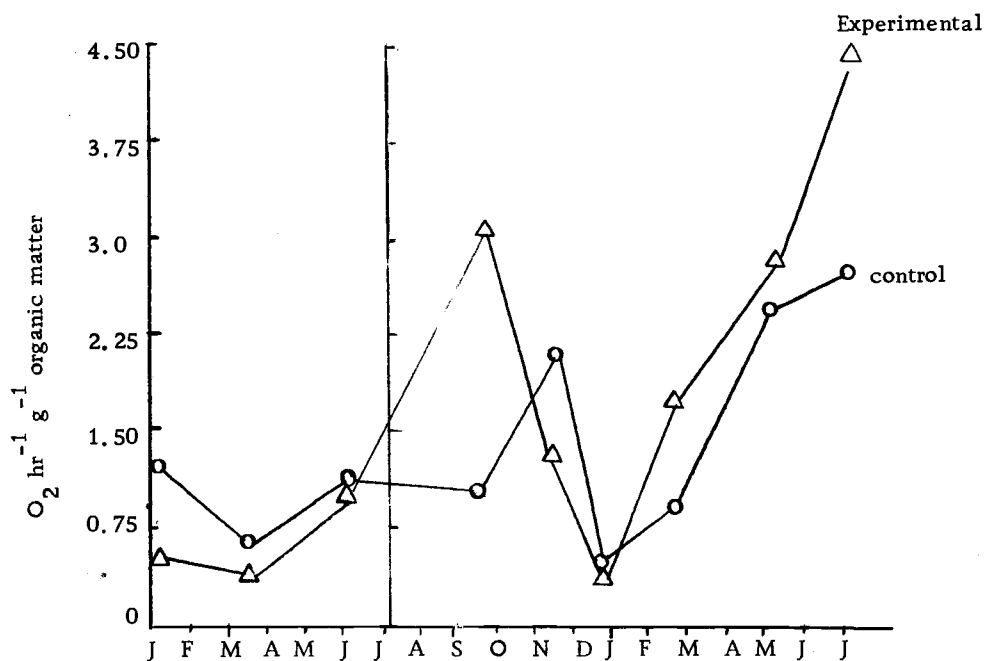


Figure 4. Rates of oxygen consumption as mg of O_2 hr^{-1} g^{-1} as measured in the P-R chamber.

Photosynthesis-Respiration Ratios

Communities from streams receiving raw effluent reached a maximum P/R ratio of 7.30 in September, 1966 which was exceeded by the maximum ratio of 8.40 of the controls the same month. Communities from experimental streams recorded a minimum P/R ratio of 2.36 in June, 1967 while the minimum for control communities of 3.7 occurred the following month (Figure 5).

A maximum P/R ratio of 12 was recorded for communities from streams receiving stabilized effluent during the month of December, 1967 exceeded by a maximum P/R ratio of 14 for control communities during the same month. The minimum P/R ratio recorded for communities from streams receiving stabilized effluent was 2.70 in April, 1968 and for control communities, 2.60 the same month.

The mean P/R ratio for communities from streams receiving raw effluent was 4.25 compared to a mean for control communities during the same period of 5.78 a difference in means significant at the 0.05 level (Table 1). Communities from streams receiving stabilized effluent averaged a P/R ratio of 6.06 while control communities averaged 5.87. Students' t-test showed the difference in means insufficient to be significant at 0.10 level (Table 1).

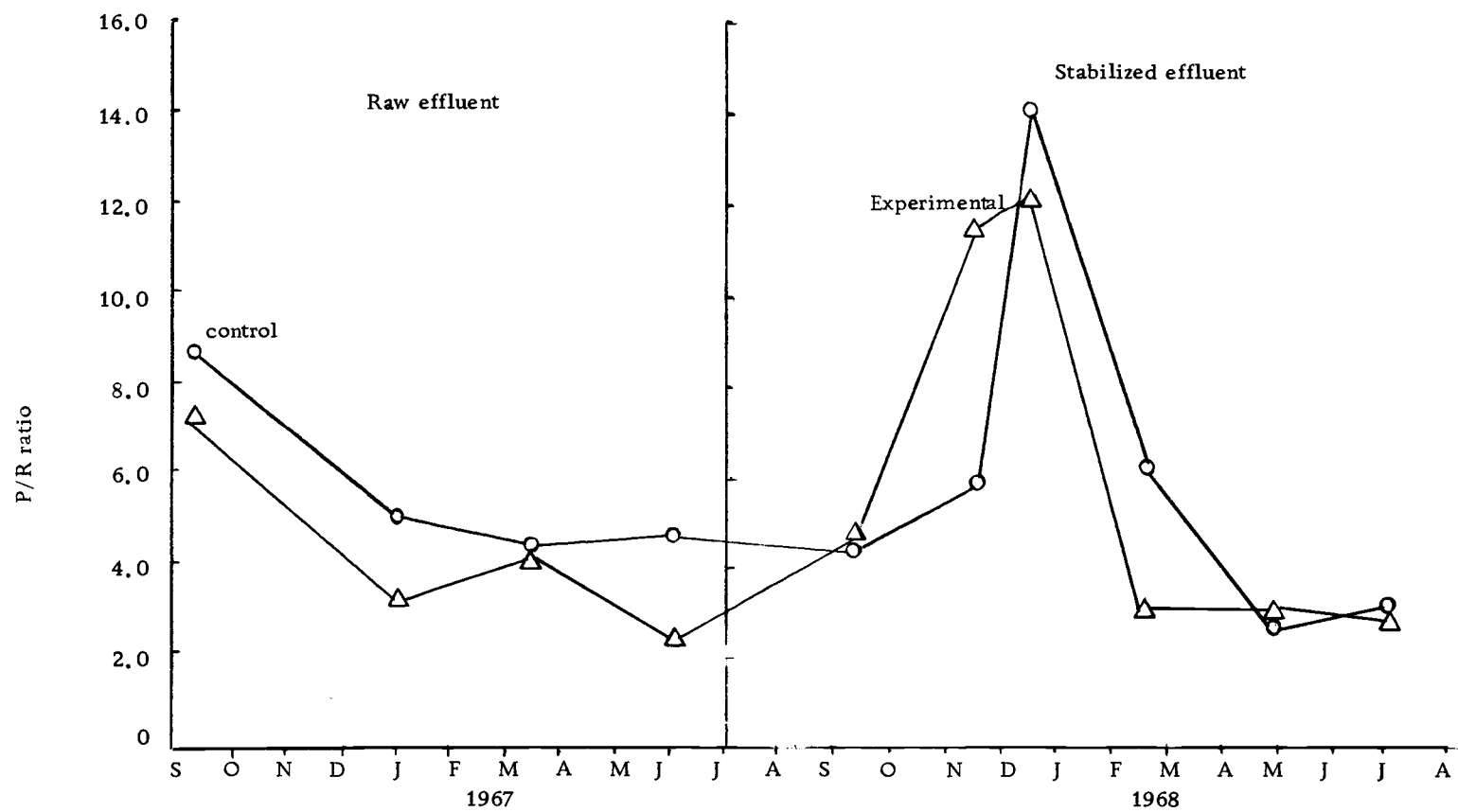


Figure 5. Ratio of photosynthesis to respiration (P/R ratio)

Biomass

Biomass expressed as ash-free dry weight of organic matter per unit area for the two year period of the experiment is plotted in Figure 6. Organic matter per unit area of communities in streams receiving raw effluent reached a maximum of 159 g m^{-2} and in control communities a maximum of 125 g m^{-2} in March, 1967. Minimum concentrations of organic matter were recorded in experimental streams of 75 g m^{-2} in January and in controls of 34 g m^{-2} in January, 1967.

The downward trend producing the July minimum concentration of organic matter in the experimental streams continued after the introduction of stabilized waste, to a two year minimum of 19 g m^{-2} in September, 1967. The minimum concentration of organic matter in the controls, 22 g m^{-2} was recorded in December, 1967.

Mean organic matter in streams receiving raw effluent was 106 g m^{-2} and in controls, 71 g m^{-2} , producing an increase in organic matter significant at the 0.10 level. The mean organic matter concentration in streams receiving stabilized effluent was 34.4 g m^{-2} and for control streams 38.7 g m^{-2} . Students' t-test failed to show a significant difference between mean organic matter per unit area in experimental and control streams in the presence of stabilized effluent (Table 1).

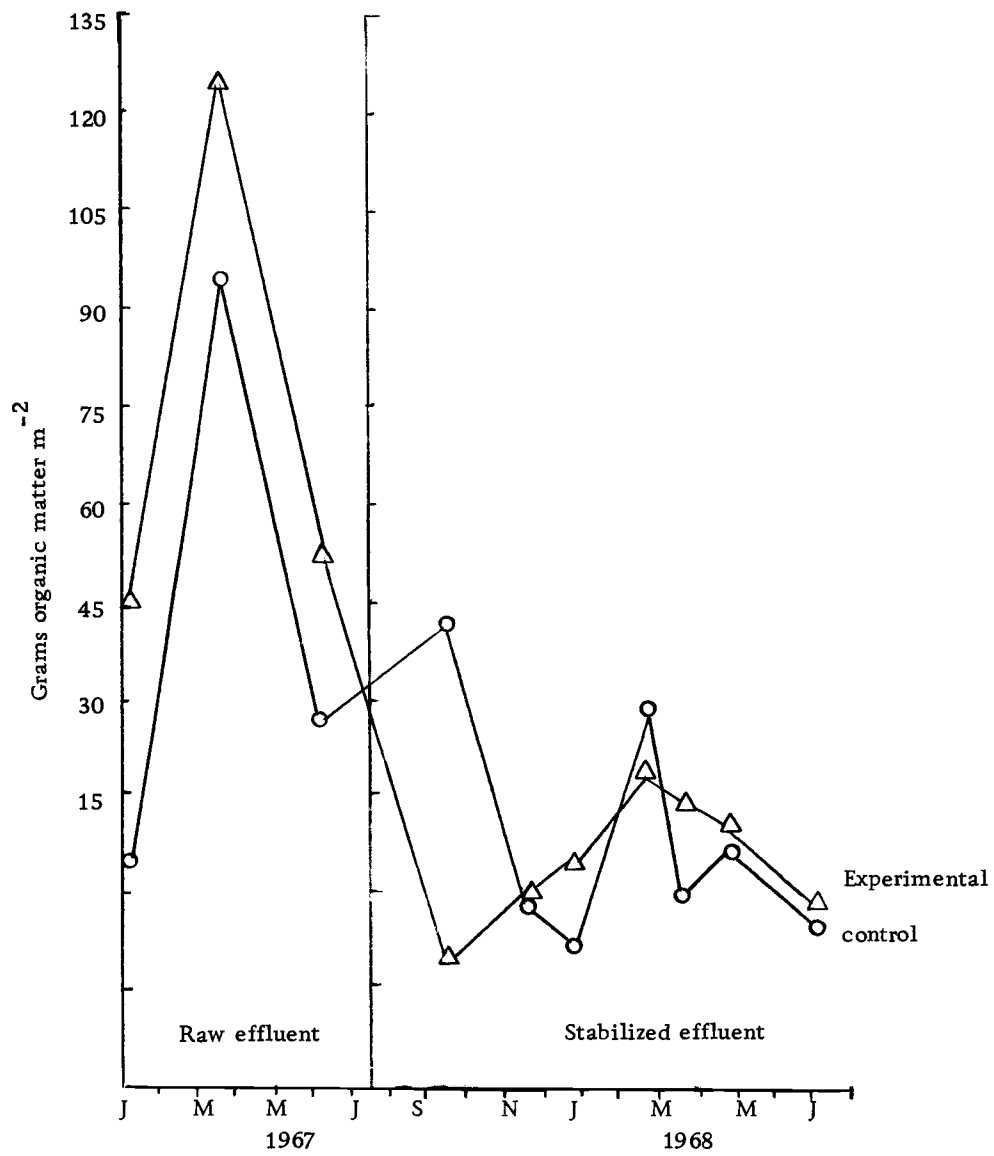


Figure 6. Biomass as ash-free dry weight of organic matter per square meter.

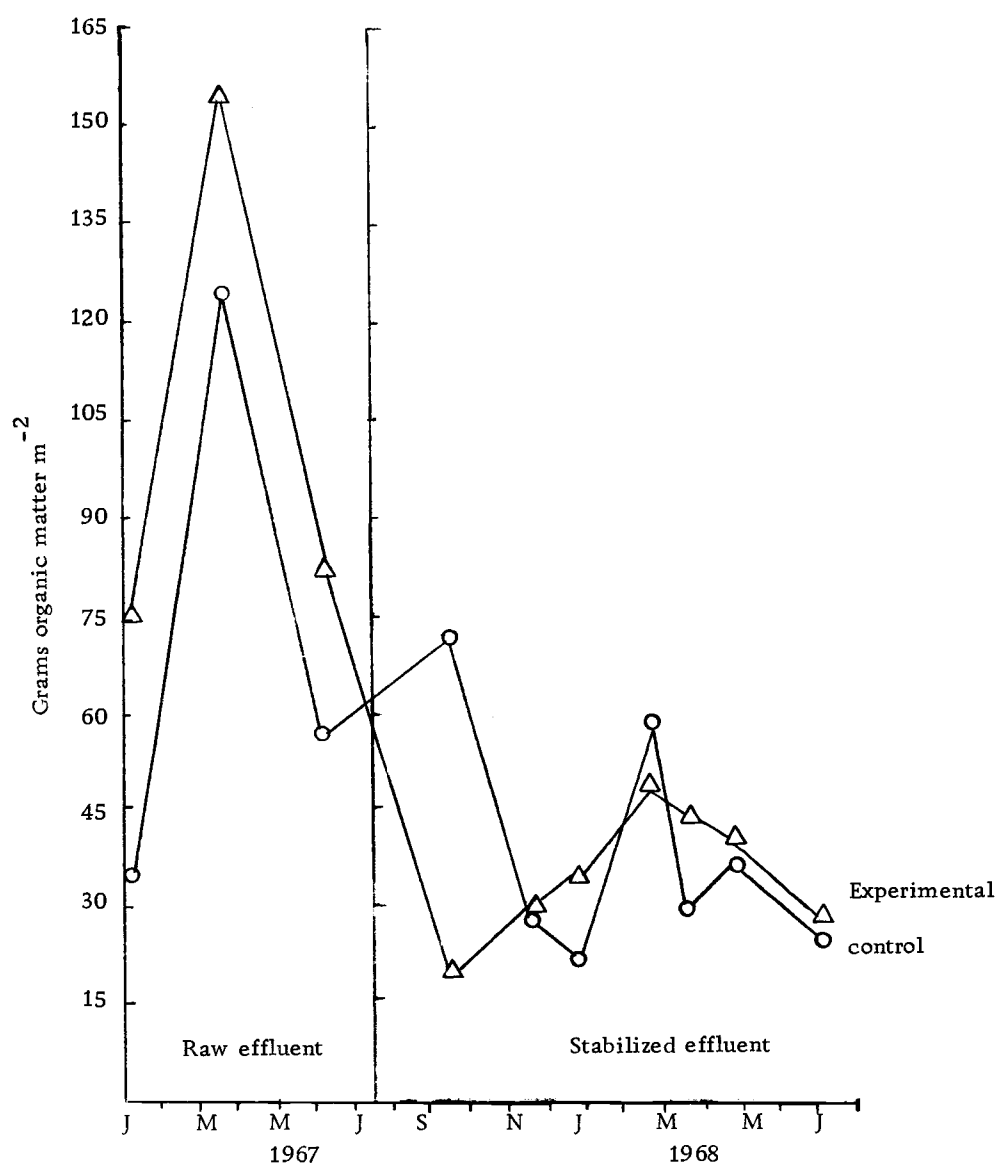


Figure 6. Biomass as ash-free dry weight of organic matter per square meter.

Concentration of Chlorophyll a Per Unit Area of Stream

The concentration of chlorophyll a m^{-2} reached a maximum value of 249 mg m^{-2} in streams receiving raw effluent and 174 mg m^{-2} in controls. Experimental streams reached their maximum concentration of chlorophyll a in January and the control streams also in January. Chlorophyll a m^{-2} in both experimental and control streams reached minimum values during June, 1967. The minimum concentration of chlorophyll a in the experimental streams was 92 mg m^{-2} and in control streams, 100 mg m^{-2} (Figure 7).

Streams receiving stabilized effluent reached a maximum concentration of chlorophyll a of 599 mg m^{-2} in March, 1968 and controls reached a maximum of 776 mg m^{-2} also in March. Chlorophyll a in experimental streams dropped to a minimum concentration of 173 mg m^{-2} in September, 1967 and the controls to a minimum of 252 mg m^{-2} in December, 1967.

Chlorophyll a m^{-2} during the first year averaged less than half that of the second year. The mean concentration during the first year when experimental streams were receiving raw effluent was 164 mg m^{-2} in experimental streams and 134 mg m^{-2} in the control streams. The second year averages were 421 mg m^{-2} in experimental streams and 423 mg m^{-2} in control streams. The differences in mean concentration of chlorophyll a in experimental and control streams

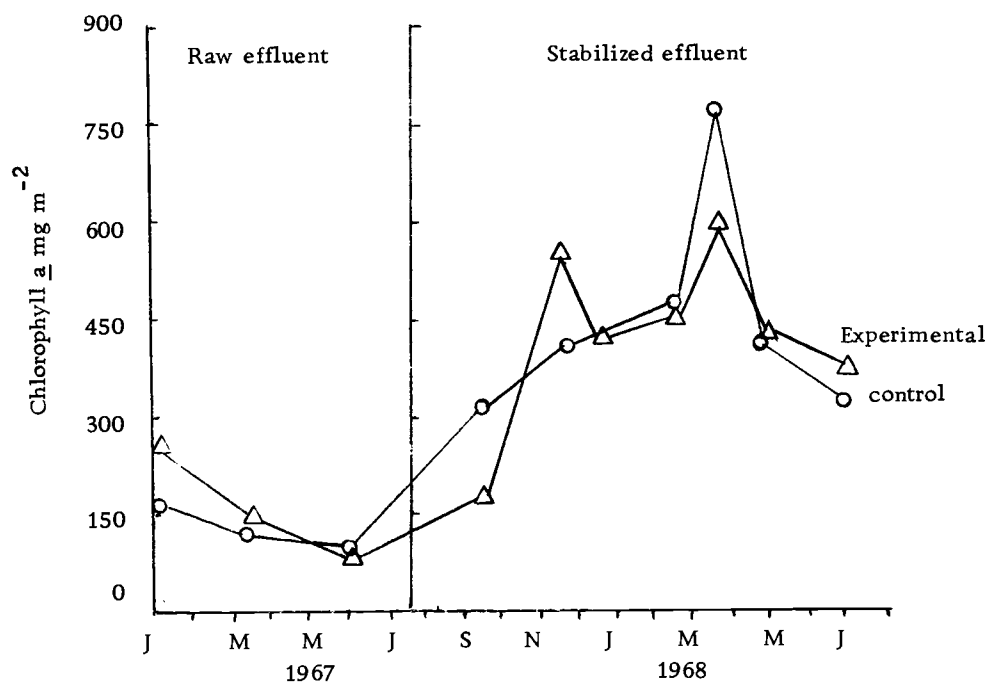


Figure 7. Concentrations of chlorophyll a per unit area.

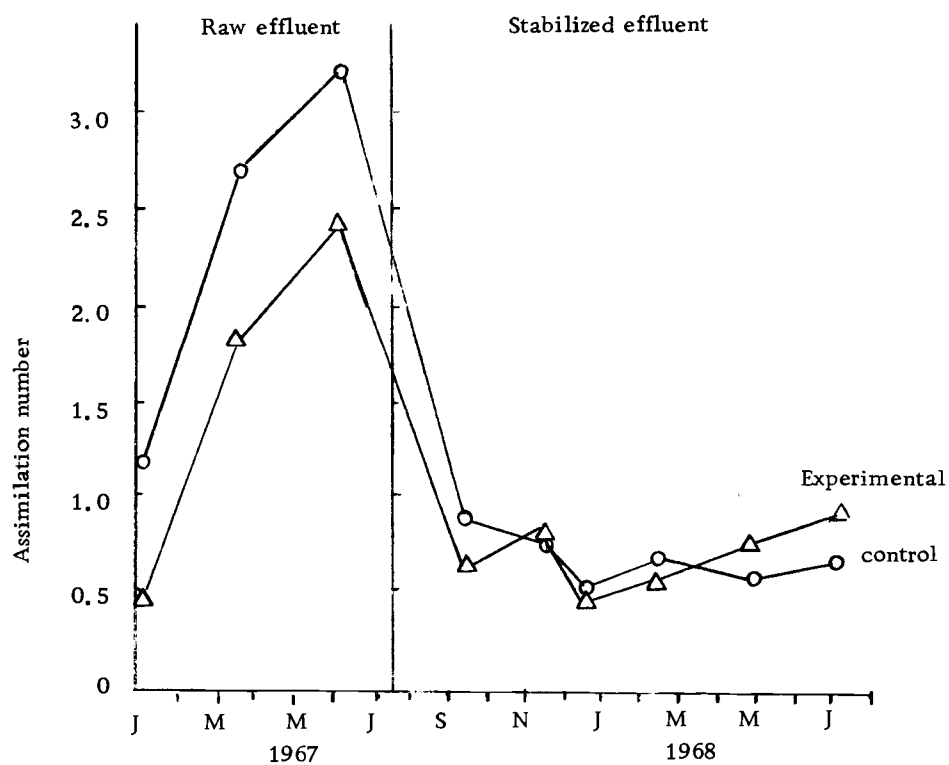


Figure 8. Assimilation number (mg O₂ hr⁻¹ mg⁻¹ chlorophyll a).

did not meet the 0.1 level of significance during either year (Table 1).

Concentration of Chlorophyll a Related to Biomass

Chlorophyll a g^{-1} of organic matter reached a maximum of 5.10 mg g^{-1} of organic matter in January in streams receiving raw effluent (Figure 9). During the same month, control streams registered a maximum of 3.3 $\text{mg chlorophyll a g}^{-1}$ of organic matter. The concentration of chlorophyll a dropped to a minimum of 0.94 mg in experimental streams and 0.99 mg g^{-1} organic matter in control streams in March, 1967.

The concentration of chlorophyll a reached a maximum of 18.7 mg g^{-1} organic matter in streams receiving stabilized effluent during the month of November, 1967 while control streams reached an earlier and higher maximum in March, 1967 of 26.4 mg . Minimum chlorophyll concentrations in both experimental and control streams occurred in September, 1967 when experimental streams registered 9.1 mg and controls 4.30 $\text{mg chlorophyll a g}^{-1}$ organic matter.

The mean concentration of chlorophyll a g^{-1} of organic matter in streams receiving raw effluent was 1.77 mg and 2.63 mg in control streams. Streams receiving stabilized effluent averaged 12.0 $\text{mg chlorophyll a g}^{-1}$ organic matter and controls during that period 9.98 mg chlorophyll . Differences between means were significant at the 0.10 level only during the second year (Table 1).

Gross Primary Production Per Milligram of Chlorophyll a

Gross primary production per milligram of chlorophyll a (assimilation number) by communities from experimental and control streams was higher during the first year than during the second, reaching a maximum of 2.40 in communities from experimental streams and 3.21 in control communities both in June (Figure 8). Minimum assimilation numbers of 0.45 in communities from experimental streams and 0.75 in control communities were recorded in January, 1967 for the former and July, 1967 for the latter.

Communities developed in streams receiving stabilized effluent recorded a maximum assimilation number of 0.90 in July, 1968 while control communities reached a maximum of 0.98 in September, 1967.

A minimum value of 0.43 was recorded for communities from experimental streams in December, 1967 and 0.56 in control communities in April, 1968.

Mean assimilation numbers of 1.55 and 0.68 in communities from streams receiving raw and stabilized waste respectively were less than those of their corresponding controls. During the first year, assimilation numbers of control communities averaged 2.36 while during the second, controls averaged 0.71. Only during the first year were the differences statistically significant (Table 1).

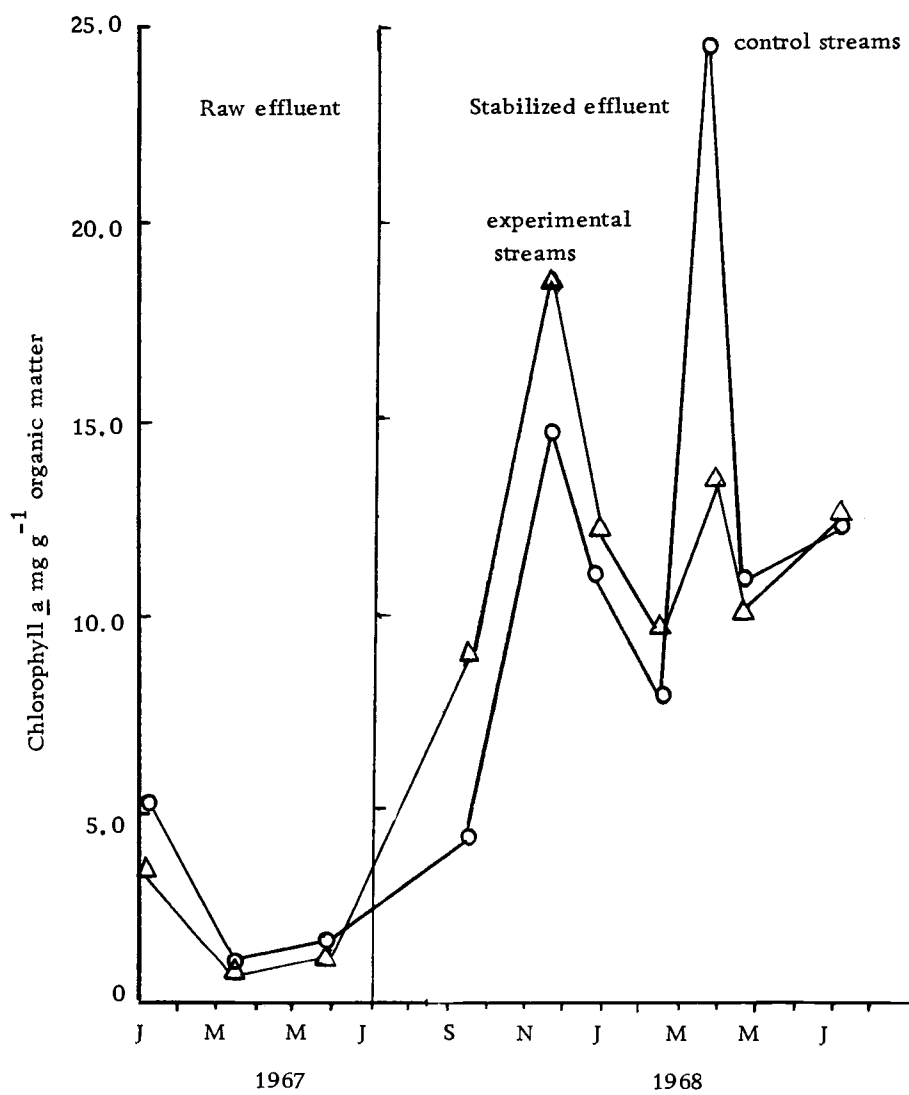


Figure 9. Chlorophyll content of the organic matter as mg chlorophyll a g⁻¹ ash-free dry weight.

Table 1. Results of Students' t-tests of significance of differences between mean values of properties of communities from control and experimental streams.

Community Property	Effluent	Period	Means		Significance P
			Control	Experimental	
Gross primary production (mg O ₂ hr ⁻¹ m ⁻²)	raw	9/66-6/67	304	238	< 0.10
Gross primary production (mg O ₂ hr ⁻¹ m ⁻²)	stab.	7/67-7/68	257	295	< 0.45
Gross primary production (mg O ₂ hr ⁻¹ g ⁻¹)	raw	1/67-6/67	4.81	1.91	< 0.10
Gross primary production (mg O ₂ hr ⁻¹ g ⁻¹)	stab.	7/67-7/68	7.17	9.82	< 0.025
Respiration (mg O ₂ hr ⁻¹ m ⁻²)	raw	9/66-6/67	52	60	< 0.20
Respiration (mg O ₂ hr ⁻¹ m ⁻²)	stab.	7/67-7/68	60	73	< 0.10
Respiration (mg O ₂ hr ⁻¹ g ⁻¹)	raw	1/67-6/67	0.97	0.66	< 0.10

Table 1 (continued)

Community Property	Effluent	Period	Means		Significance P
			Control	Experimental	
Respiration (mg O ₂ hr ⁻¹ g ⁻¹)	stab.	7/67-7/68	1.64	2.33	< 0.05
P/R ratio	raw	9/66-6/67	5.78	4.25	< 0.05
P/R ratio	stab.	7/67-7/68	5.87	6.06	< 0.45
Organic matter (g m ⁻²)	raw	1/67-6/67	71	106	< 0.10
Organic matter (g m ⁻²)	stab.	7/67-7/68	38.7	34.4	< 0.25
Chlorophyll <u>a</u> (mg m ⁻²)	raw	1/67-6/67	134	164	< 0.15
Chlorophyll <u>a</u> (mg m ⁻²)	stab.	7/67-7/68	423	421	< 1
Chlorophyll <u>a</u> (mg g ⁻¹ organic matter)	raw	1/67-6/67	2.63	1.77	< 0.20
Chlorophyll <u>a</u> (mg g ⁻¹ organic matter)	stab.	7/67-7/68	9.98	12.0	< 0.10
Assimilation number	raw	1/67-6/67	2.36	1.55	< 0.10
Assimilation number	stab.	7/67-7/68	0.71	0.68	< 1

Effect of Effluent on Community Metabolism
in the P-R Chamber

Stabilized Kraft effluent from mill A was added to intact communities in the P-R chamber on four occasions during photosynthesis-respiration measurements in the fall and winter of 1967-68. Rates of net photosynthesis and respiration were measured and compared to measurements taken prior to adding the effluent and subsequent to complete removal of effluent by exchanging the water in the chamber. If the rate of respiration or photosynthesis after measurements in the presence of the effluent differed by more than 10% from that prior to measurement in the presence of the effluent, it was assumed the community had begun to change in character and that observation was discarded.

In making statistical comparisons, data from observations on communities from control and experimental streams were lumped to increase the number of observations included in Students' t-test of significance. Respiration appeared to be enhanced by the presence of Kraft mill effluent with a t-test significance of 0.025. No statistically significant change in production occurred during exposure to the effluent. However, all the measurements under exposure at the 4% concentration of effluent produced an increase in gross production (Table 6).

Table 2. The effect of Kraft mill effluent on production of respiration of communities developed in laboratory streams as measured on the P-R chamber. Photosynthesis measured under 9,150 lux illumination. Kraft mill effluent concentration of 1.5% used on Sept. 15, 4% for other experiments. O_2 /hr/two trays (mg). Students' t-test of significance.

Date	BOD	Respiration		Net production		Gross production	
		no effluent	with effluent	no effluent	with effluent	no effluent	with effluent
Sept. 15, 1967	7						
Control		16	--	51	51	57	--
Experimental		13	15	48	43	61	58
Nov. 20, 1967	2						
Control		13	24	68	67	81	91
Experimental		9	--	96	88	105	--
Dec. 20, 1967	31						
Control		3	4	35	38	38	42
Experimental		--	--	--	--	--	--
Feb. 19, 1968	14						
Control		12	15	61	65	73	80
Experimental		9	14	35	45	49	59
Mean		10	14.4	56	57	60	66
Level of significance		$P \leq 0.025$		$P \leq 0.45$		$P \leq 0.15$	

Effluent from Stabilization Pond of Mill B

From July 5 to October 25, 1969 experimental streams received effluent from the stabilization pond of mill B. Data collected during this period are presented in Table 3 and represented graphically in Figures 10 and 11.

Organic matter in control streams increased between July 5 and October 25 from 25.3 to 69 g m⁻². During this same period, the streams receiving 40 ml of effluent per liter of water registered a decline in organic matter from 28.3 g m⁻² to 22 g m⁻². On September 13, communities developed in a series of concentrations of effluent from Mill B were sampled. The second lowest concentration of organic matter, 31 g m⁻² occurred in the stream receiving 5 ml of effluent per liter of water. However, the other communities registered a diminishing biomass with increasing concentration of effluent from 59 g in the control stream to 28 g m⁻² in the stream receiving the highest concentration of effluent.

The concentration of chlorophyll a g⁻¹ of organic matter was remarkably constant from April, prior to the beginning of application of effluent from mill B until the end of the experiment. As a result, the concentration of chlorophyll a per unit area correlated closely with organic matter per unit area. Again, the stream receiving 5 ml of effluent per liter of water produced an anomalous result, yielding

Table 3. Organic matter (ash-free dry weight) and chlorophyll a concentrations of communities developed in streams at different concentrations of effluent from stabilization pond of mill B.

Characteristic	Control	5 ml/l	10 ml/l	20 ml/l	40 ml/l
Sept. 3, 1968					
g organic matter m ⁻²	59	31	41	38	28
mg chlorophyll <u>a</u> m ⁻²	711	270	477	427	304
mg chlorophyll <u>a</u> g ⁻¹ organic matter	12.1	8.7	11.6	11.2	10.9
Oct. 25, 1968					
g organic matter m ⁻²	69				22
mg chlorophyll <u>a</u> m ⁻²	890				291
mg chlorophyll <u>a</u> g ⁻¹ organic matter	12.9				13.2

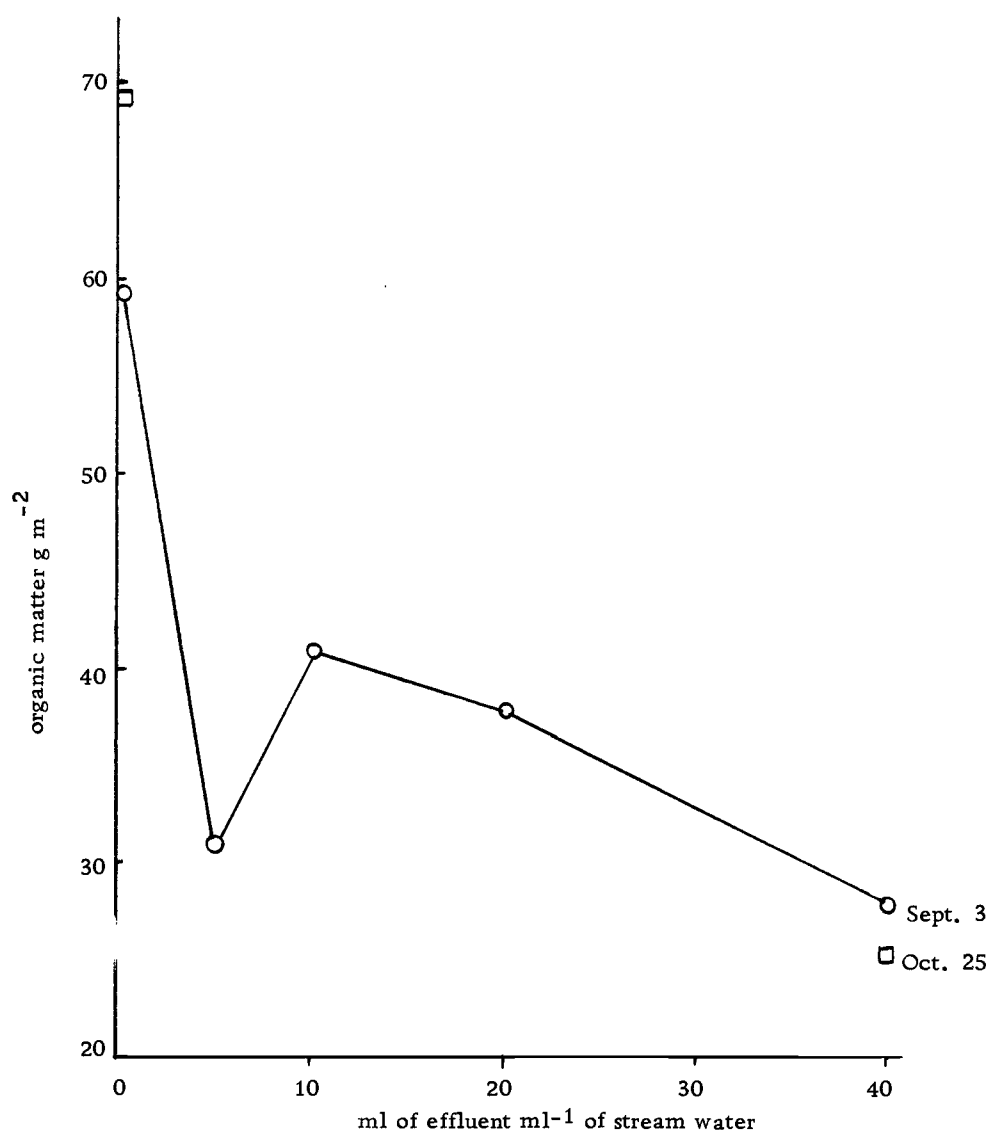


Figure 10. Concentration of organic matter as a function of the ratio of effluent from the stabilization pond of mill B to water entering laboratory streams.

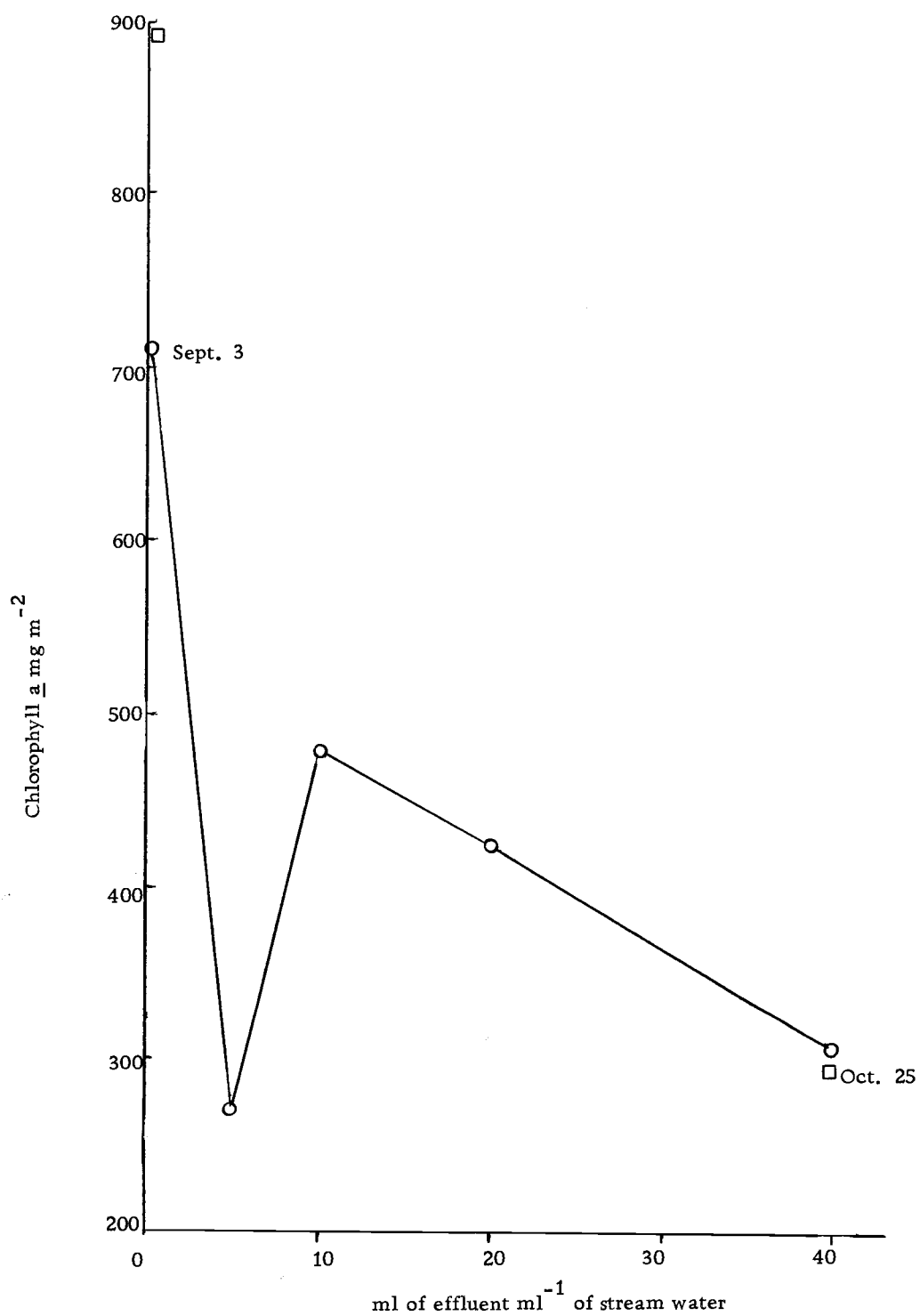


Figure 11. Concentration of chlorophyll *a* as a function of the ratio of effluent from the stabilization pond of mill B to water entering laboratory streams.

only 8.7 mg of chlorophyll a per gram of organic matter in contrast to values ranging between 10.9 and 13.2 mg g⁻¹ of organic matter from the other streams.

Because two substrate trays were necessary to make adequate measurements in the P-R chamber and only one stream was available for each of the four concentrations applied from July 5 to September 3, data for photosynthesis and respiration were not obtained September 3. However, since the chlorophyll a per unit mass of organic matter, assimilation number, and P/R ratios remained relatively constant during the previous months, estimates based upon them are possible.

Caloric Content

Caloric content of control and experimental stream communities did not differ significantly during the two years of the research, averaging 4500 calories per gram of organic matter.

Community Structure

Densities of the Diatom Populations

Densities of dominant species of diatoms observed at each sampling interval are presented in tabular form in the Appendix Table 10. Mean densities of populations of species whose densities

differed significantly between control and experimental streams during the term of each experimental treatment and the results of Students' t-test of significance of contrast are presented in Table 4.

Navicula (sp.) includes three quite similar species, N. cryptocephala, N. radiosa, and N. rhyncocephala. Since Navicula cryptocephala was by far the most common and the other two relatively rare, it was expedient to lump them for purposes of counting.

Density data has been plotted in Figures 12 through 17 for those species in which significant differences in mean density in experimental and control streams occurred over the two year period. Only those species which showed a consistently higher density in either the control or experimental streams were considered. No effort was made to demonstrate a difference by statistical methods unless a clear pattern was evident throughout all or most observations.

Navicula (sp.) showed significant differences in mean population densities between experimental and control streams in the presence of raw effluent from mill A (Table 4). Maximum densities of 3.69×10^9 cells per square meter were present in experimental streams and 1.45×10^9 per square meter in control streams in January, 1967. Minimum population densities of 7.15×10^8 cells per square meter present in June, in both control and experimental streams. The mean density for experimental streams during this period was 3.20×10^9 while that of the controls was 2.43×10^9 cells

per square meter. Students' t-test indicated a significance of 0.025 for this difference in means.

Population density of Synedra ulna in streams receiving raw effluent exceeded that recorded for control streams at each sample period. Maximum density recorded for experimental streams during this period was in March when the population reached a density of 5.97×10^9 cells per square meter as compared to a maximum of 4.81×10^8 on the same date in the control streams (Figure 12).

Streams receiving stabilized waste from mill A and waste from the stabilization pond of mill B, consistently supported smaller populations of Synedra ulna than did the control streams. Experimental streams recorded a maximum population density of 3.6×10^8 per square meter in April, 1968 and a minimum density of 1.38×10^7 cells in December, 1967 during introduction of stabilized effluent from mill A. Maximum density in control streams during this period was 3.39×10^9 cells per square meter in November, 1967 and minimum density 4.1×10^7 in April, 1968.

The mean densities were 3.86×10^9 and 3.8×10^8 cells per square meter in experimental and control streams respectively during the first period, a difference significant at the 0.005 level. The second period of treatment when experimental streams were receiving stabilized effluent from mill A produced means of 1.5×10^8 and 1.3×10^9 cells per square meter for experimental and control

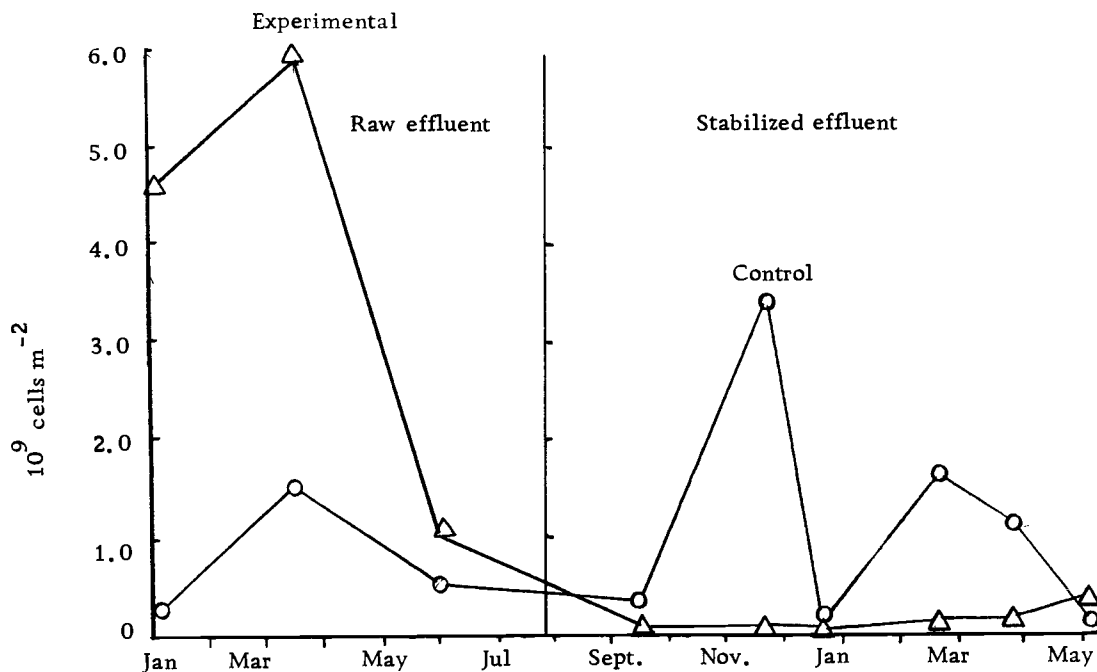


Figure 12. Comparison of cell densities of *Synedra ulna* in control and experimental streams as related to time and the nature of the effluent from mill A.

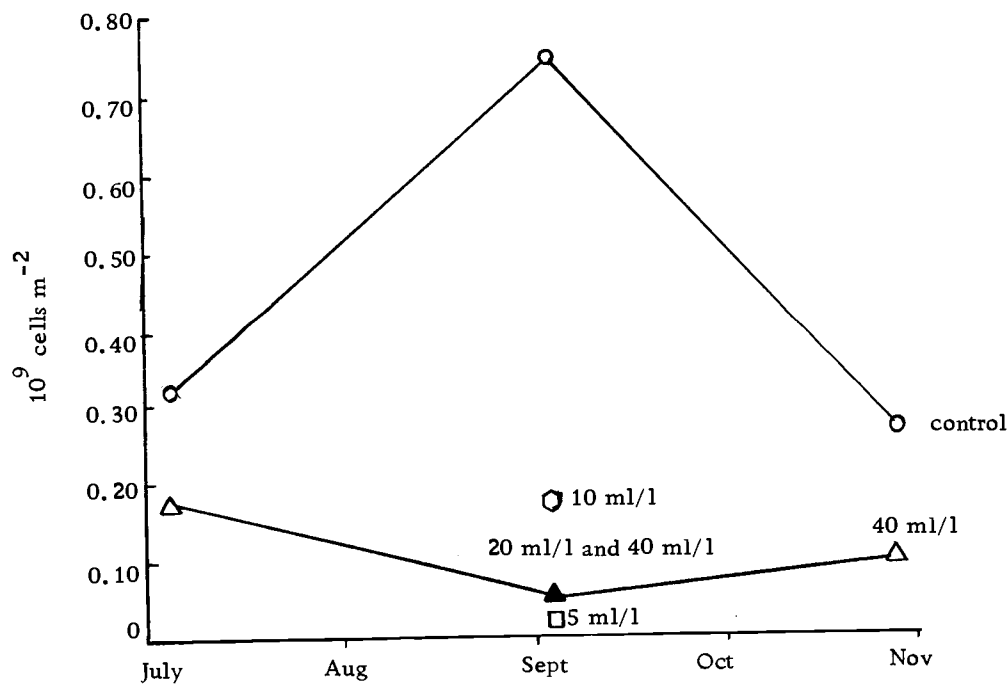


Figure 13. Comparison of cell densities of *Synedra ulna* in control and experimental streams as related to time and the concentration of effluent from mill B.

streams respectively (Table 4).

Rhoicosphenia curvata reached maximum density in June in experimental streams receiving raw effluent of 9.65×10^8 cells per square meter (Figure 14). Maximum density in control streams during this time reached 2.01×10^{10} cells in January, 1967. Minimum densities occurred in both experimental and control streams in March, 1967 when R. curvata was absent from experimental streams and was present at a density of 1.09×10^9 cells per square meter in control streams.

The mean density for this species in the experimental streams during this period was 4.0×10^8 and 9.47×10^9 cells per square meter in experimental and control streams respectively. The difference between these means was significant at the 0.025 level (Table 4).

The maximum population density of R. curvata in experimental streams during treatment with stabilized effluent from mill A of 8.11×10^9 cells per square meter occurred in November, 1967 (Figure 14). Minimum density of this taxon was recorded in streams receiving stabilized effluent from mill A of 3.59×10^9 cells per square meter in March, 1968. The maximum density of cells in control streams during this time was 3.66×10^9 cells per square meter in July, 1968 and the minimum was 4.28×10^8 in November, 1967. The mean density was 6.08×10^9 cells per square meter for

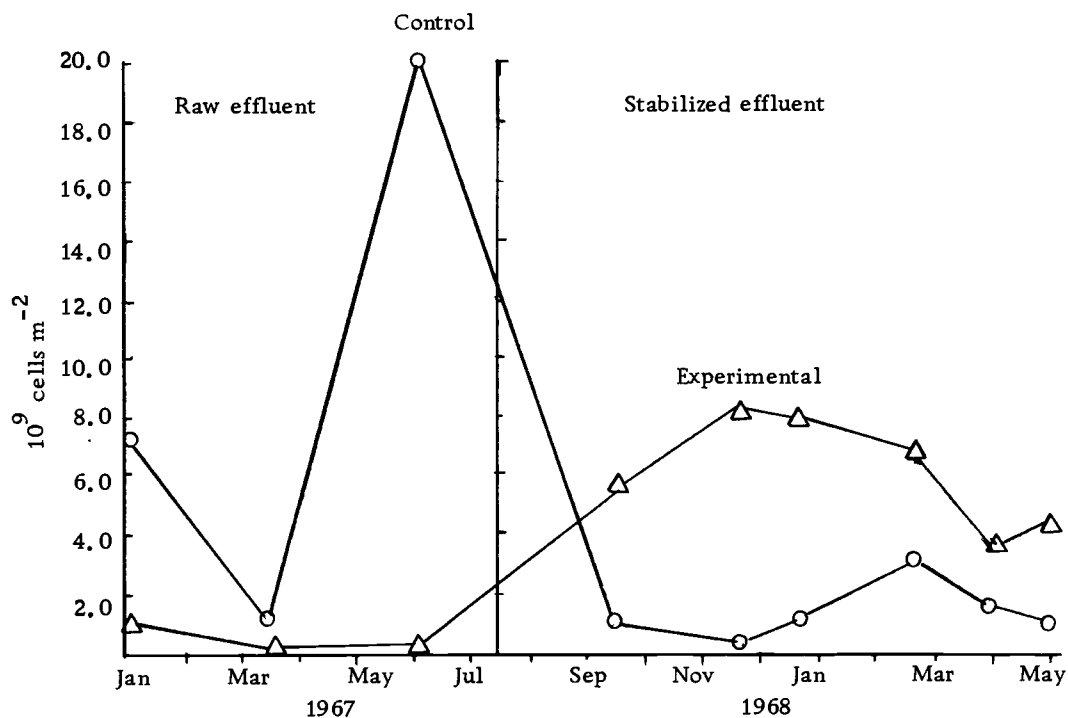


Figure 14. Comparison of cell densities of *Rhoicosphenia curvata* in control and experimental streams as related to time and the nature of the effluent from mill A.

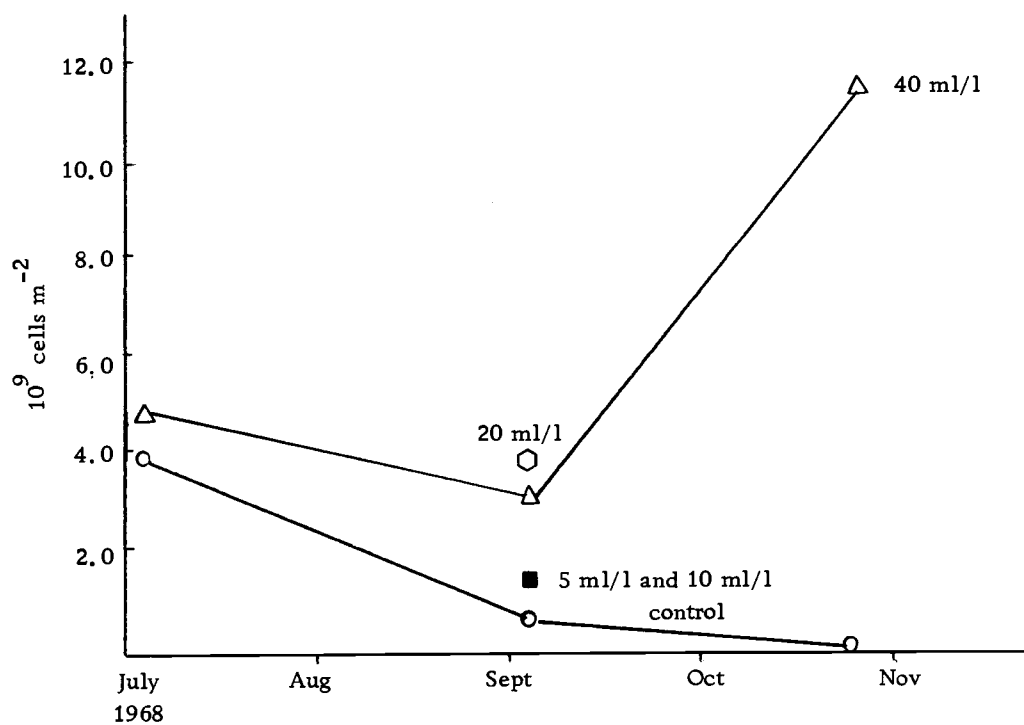


Figure 15. Comparison of cell densities of *Rhoicosphenia curvata* in control and experimental streams as related to time and the concentration of effluent.

experimental streams receiving stabilized effluent from mill A and 1.45×10^9 in controls (Table 4).

A consistent difference in population density of Achnanthes minutissima was apparent only when streams receiving raw effluent were compared with control streams. Mean population density of this taxon in experimental streams was 6.55×10^9 cells while that in control streams was 9.38×10^9 cells per square meter, a difference significant at the 0.05 level (Table 4).

Densities ranged from 1.25×10^{10} to 1.76×10^9 cells per square meter in January and June respectively in experimental streams and from 1.24×10^{10} to 6.04×10^9 cells per square meter for the same dates in control streams.

Significant differences between population densities of Melosira varians in control and experimental streams appeared during treatment with stabilized waste (Figure 16). Means of 3.25×10^9 and 1.35×10^9 cells per square meter were recorded in experimental and control streams respectively during application of stabilized waste from mill A. The difference between the means was significant at the 0.01 level (Table 4).

Fragilaria brevistriata first appeared in August, 1967 in control streams increasing in abundance to a maximum density of 3.4×10^{10} cells per square meter during July, 1968 (Figure 18). Streams receiving stabilized effluent from mill A reached a

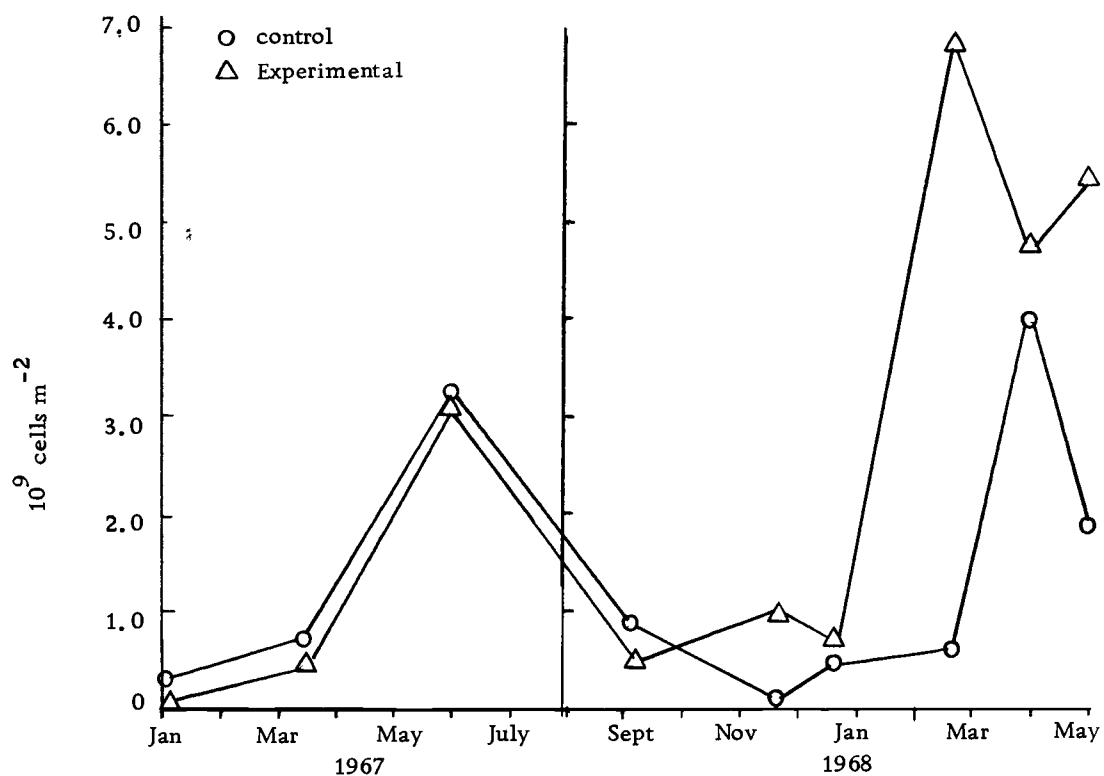


Figure 16. Comparison of cell densities of *Melosira varians* in control and experimental streams as related to time and the nature of the effluent from mill A.

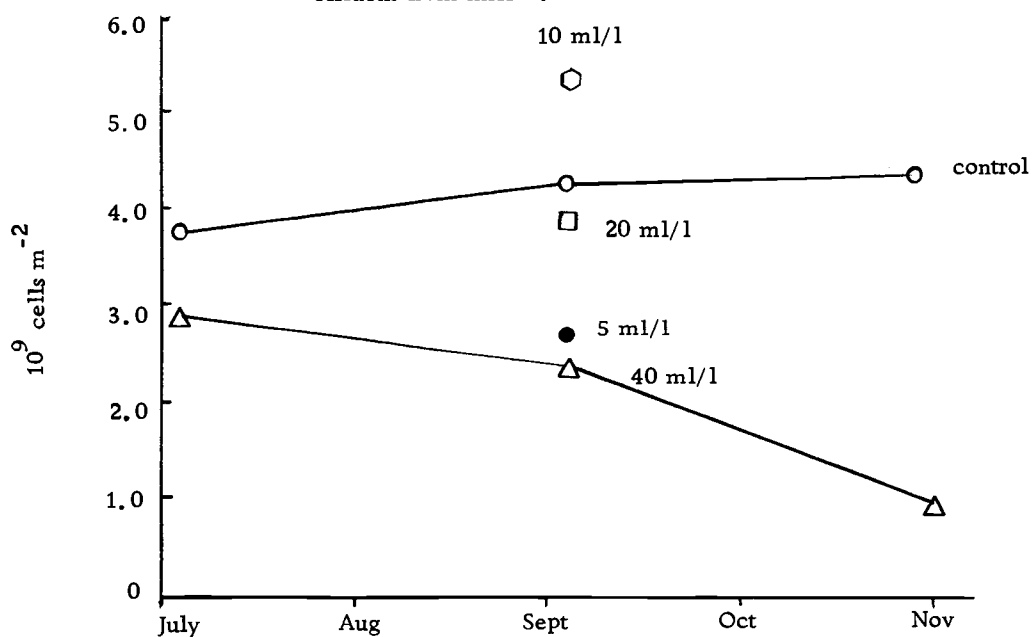


Figure 17. Comparison of cell densities of *Melosira varians* in control and experimental streams as related to time and the concentration of effluent.

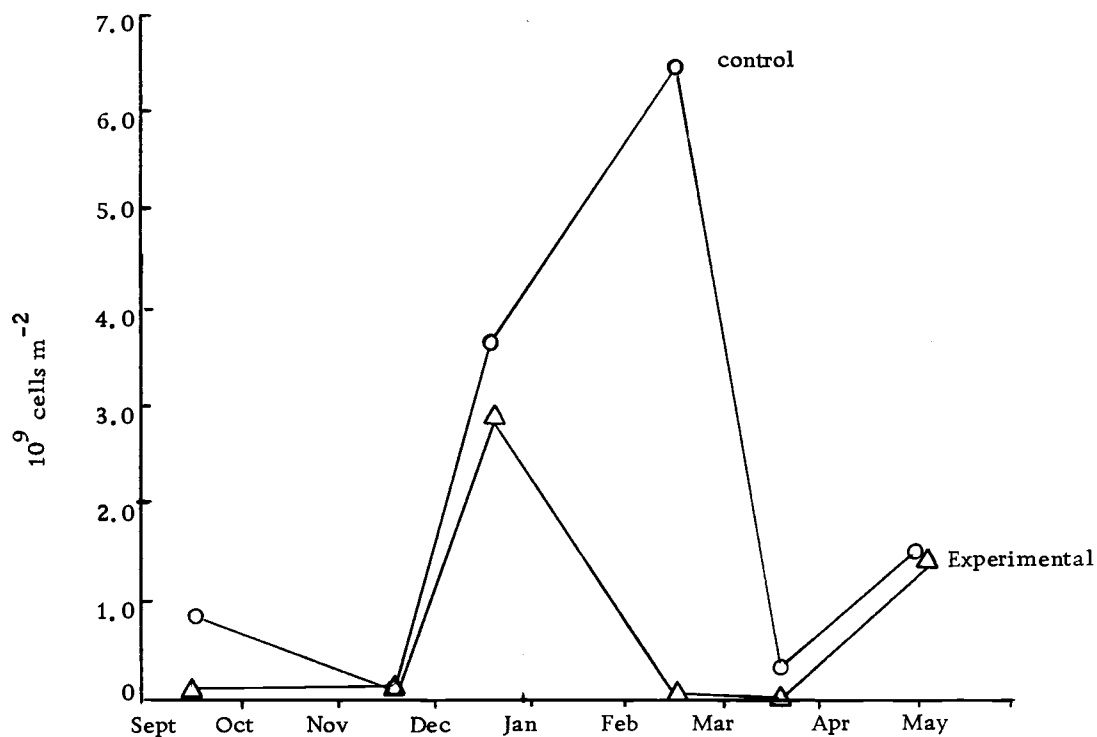


Figure 18. Comparison of cell densities of *Fragilaria brevistriata* in control and experimental streams as related to time and the nature of the effluent from mill A.

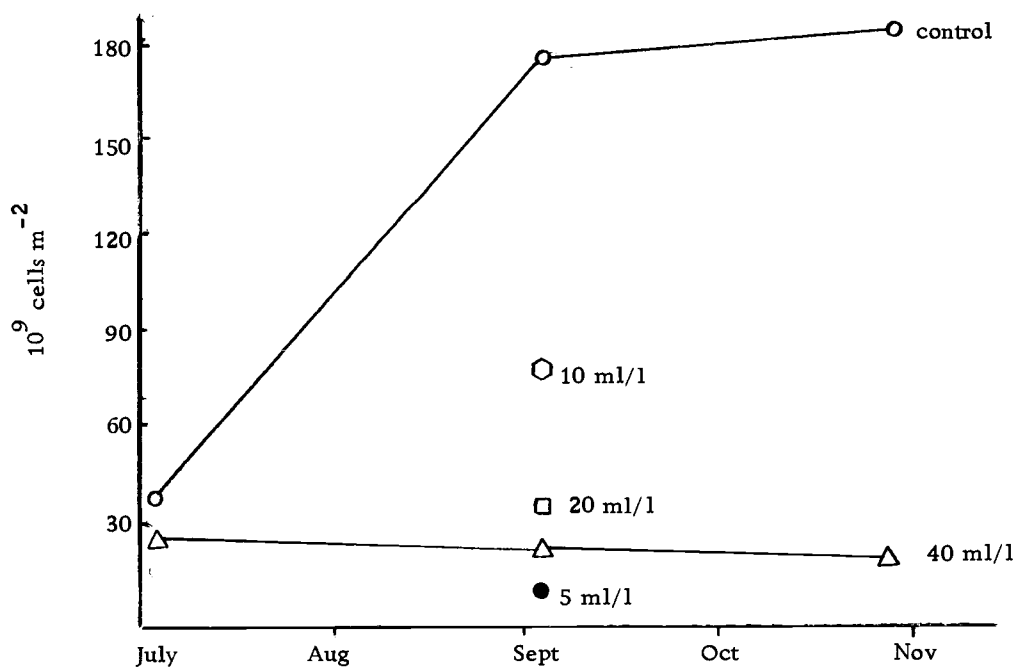


Figure 19. Comparison of cell densities of *Fragilaria brevistriata* in control and experimental streams as related to time and the concentration of effluent.

population density of F. brevistriata of 2.31×10^{10} cells per square meter in July, 1968. Mean densities in experimental and control streams during the period when experimental streams were receiving stabilized effluent from mill A were 7.2×10^8 and 2.11×10^8 cells per square meter respectively. The difference between the means was significant at the 0.025 level (Table 4).

Cocconeis placentula var. euglypta registered significant differences in mean population densities between experimental and control streams during treatment with both raw and stabilized effluent from mill A. In the presence of raw effluent, the mean density of C. placentula was 5.1×10^8 cells compared to a density of 2.09×10^9 cells per square meter in the controls (Table 4).

When stabilized waste was added to experimental streams, the relative position of the two means was reversed, experimental streams supporting a mean population density of 3.13×10^9 cells and the control streams a mean density of 1.7×10^9 cells per square meter, with a significant difference between the means in both cases at the 0.005 level.

The mean density of the total diatom population during the introduction of raw effluent was 5.6×10^{10} cells in experimental streams and 4.3×10^{10} cells per square meter in control streams (Figure 2). Population density in experimental streams ranged from 1.44 to 11×10^{10} cells per square meter. In control streams the densities

Table 4. Results of Students' t-test of significance of differences between mean densities of diatom populations meeting the 0.10 level of significance. (10^9 cells m^{-2})

Diatom	Effluent	Period	Mean		t-statistic	Conclusion
			Control	Experimental		
Navicula (sp.)	Raw	1/67-6/67	0.96	2.43	3.20	$M_2 > M_1$
Synedra ulna	Raw	1/67-6/67	0.38	3.86	4.72	$M_2 > M_1$
Synedra ulna	Stabilized	8/67-5/68	1.30	0.15	3.05	$M_1 > M_2$
Rhoicosphenia curvata	Raw	1/67-6/67	9.47	0.40	3.23	$M_1 > M_2$
R. curvata	Stabilized	8/67-5/68	1.45	6.08	10.8	$M_2 > M_1$
Fragilaria brevistriata	Stabilized	8/67-5/68	2.11	0.72	2.44	$M_1 > M_2$
Cocconeis placentula	Raw	1/67-6/67	2.09	0.51	5.14	$M_1 > M_2$
C. placentula	Stabilized	8/67-5/68	1.7	3.13	9.06	$M_2 > M_1$
Achnanthes minutissima	Raw	1/67-6/67	9.38	6.55	2.28	$M_1 > M_2$
Melosira varians	Stabilized	8/67-5/68	1.35	3.25	2.97	$M_2 > M_1$

ranged from 3.18 to 5.2×10^{10} cells per square meter (Figure 20).

During the second year, when stabilized effluent from mill A was being introduced into the streams, mean total diatom population density was 2.44×10^{10} cells in experimental streams compared to 3.61×10^{10} cells per square meter in control streams. The range in density for the former was 1.56 to 3.78×10^{10} cells and in the latter, 8.10×10^9 to 1.21×10^{11} diatoms per square meter (Figure 21).

The diatom Gomphoneis herculeana appeared in small numbers in control streams during the spring and summer months from March to September of 1967 and again briefly during September and October of 1968. However, a single specimen was noted on only one occasion in the experimental streams.

Relative Frequency of Filamentous Species

Because of the difficulty in making accurate estimates of absolute population densities of filamentous Chlorophyta, control and experimental streams are compared on the basis of presence or absence in a given number of microscope fields as described by McIntire (1966).

The method consists of placing aliquants representing a standard proportion of the total harvest of either fresh or preserved material on slides and recording presence or absence in each field

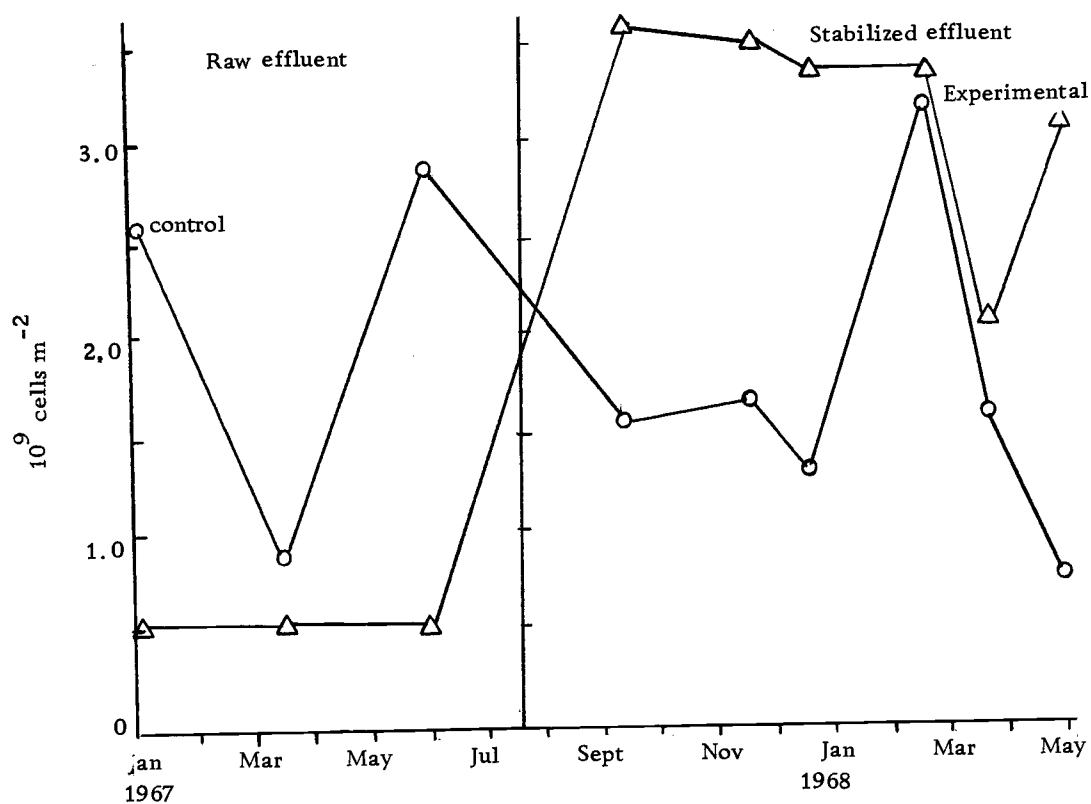


Figure 20. Comparison of cell densities of *Cocconeis placentula* var. *euglypta* in control and experimental streams as related to time and the nature of the effluent from mill A.

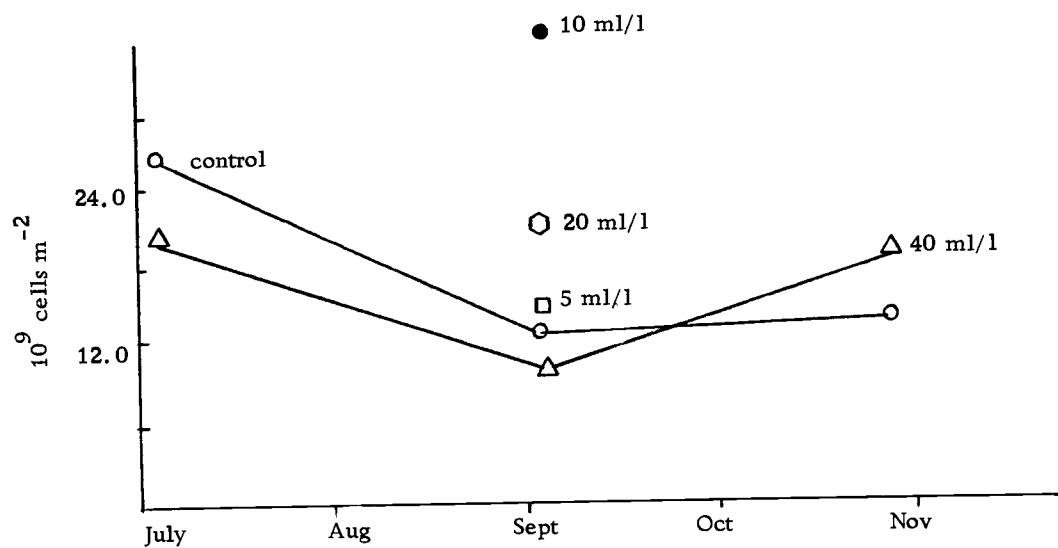


Figure 21. Comparison of cell densities of *Cocconeis placentula* var. *euglypta* in control and experimental streams as related to time and the concentration of effluent.

without regard to the number of cells per field. Results are expressed in terms of the percent of the fields viewed in which the taxon is present. In the compilation of these data, 60 fields were examined on each slide instead of the 30 fields examined by McIntire.

Three filamentous species were sufficiently abundant to permit comparisons. Oedogonium sp. Stigeoclonium subsecundum and the Chantrelle stage of the fresh-water red alga Batrachospermum showed no consistent difference in abundance between experimental and control streams (Tables 5 and 6). S. subsecundum did not persist in any of the streams after the light intensity was reduced in March, 1967 while Batrachospermum did not appear until July, 1968, disappearing again by October, 1968.

The greatest abundance of Oedogonium was in February, 1968 when it occurred in 88% of the microscope fields of samples taken from streams receiving stabilized effluent from mill A and 60% of the microscope fields of slides from control streams.

Despite the fact that Oedogonium did not occur consistently with higher frequency during treatment with stabilized effluent, the mean frequency in the experimental streams was 68.5% compared to 55.3% in the controls. The Students' t-test indicated the difference between means to be significant at the 0.025 level.

S. subsecundum reached maximum abundance in March, 1967 when it appeared in 42% of microscope fields from experimental

Table 5. Percentage of 60 microscope fields in which Oedogonium sp. and Stigeoclonium subsecundum were observed. Effluent from Mill A.

Date	<u>Oedogonium</u> sp.		<u>Stigeoclonium subsecundum</u>	
	Control	Experimental	Control	Experimental
RAW EFFLUENT				
Jan. 3, 1967	0	0	17	28
March 15, 1967	2	7	53	42
June 6, 1967	38	2	0	0
STABILIZED EFFLUENT				
Sept. 15, 1967	53	23	0	0
Nov. 20, 1967	48	80	0	0
Dec. 20, 1967	53	52	0	0
Feb. 19, 1968	60	88	0	0
March 20, 1968	58	82	0	0
April 30, 1968	60	86	0	0
July 5, 1968	57	50*	0	0

*Received 40 ml effluent per liter of water

Table 6. Percentage of 60 microscope fields in which Oedogonium sp. and Batrachospermum sp. were observed. Effluent from stabilization pond of Mill B.

Concentration	<u>Oedogonium</u> sp.		<u>Batrachospermum</u> sp.	
	Sept. 3	Oct. 28	Sept. 3	Oct. 28
Control	8	5	12	0
5 ml/l	17	--	10	--
10 ml/l	3	--	8	--
20 ml/l	23	--	15	--
40 ml/l	30	23	18	0

streams treated with raw effluent and 53% of the control fields.

By June, it had completely disappeared.

Batrachospermum was present on only two sampling dates, July 5 and September 3, 1968 with a maximum frequency on the latter date of 18% in experimental communities and 12% of the fields viewed in control communities.

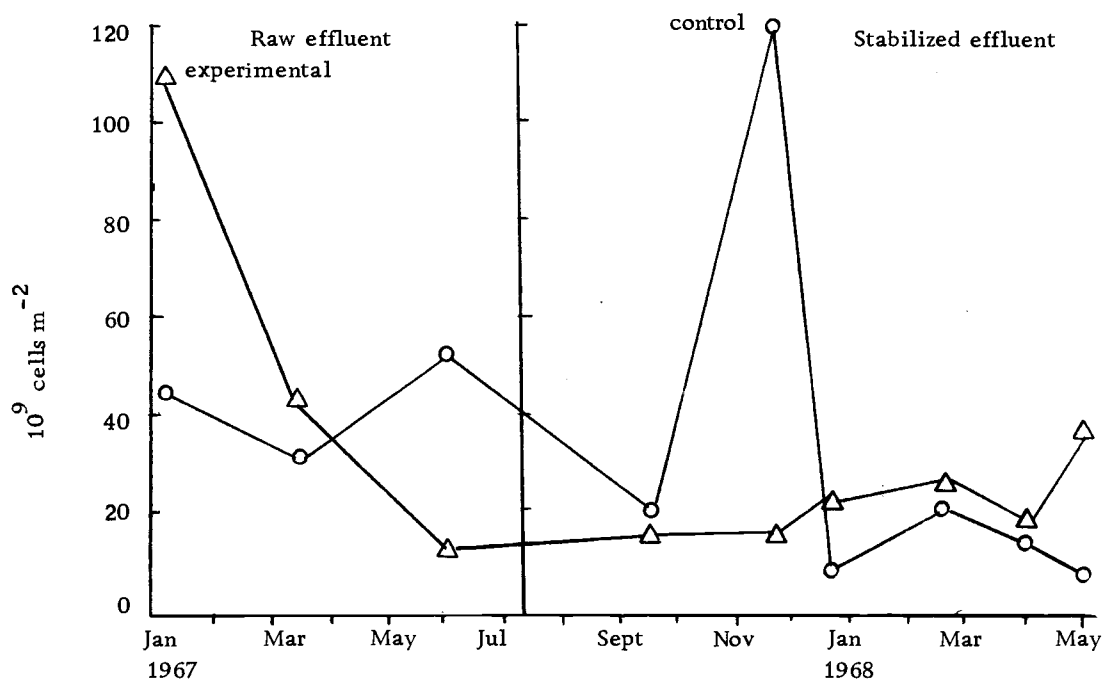


Figure 22. Comparison of total diatom cell densities in control and experimental streams as related to time and the nature of the effluent from mill A.

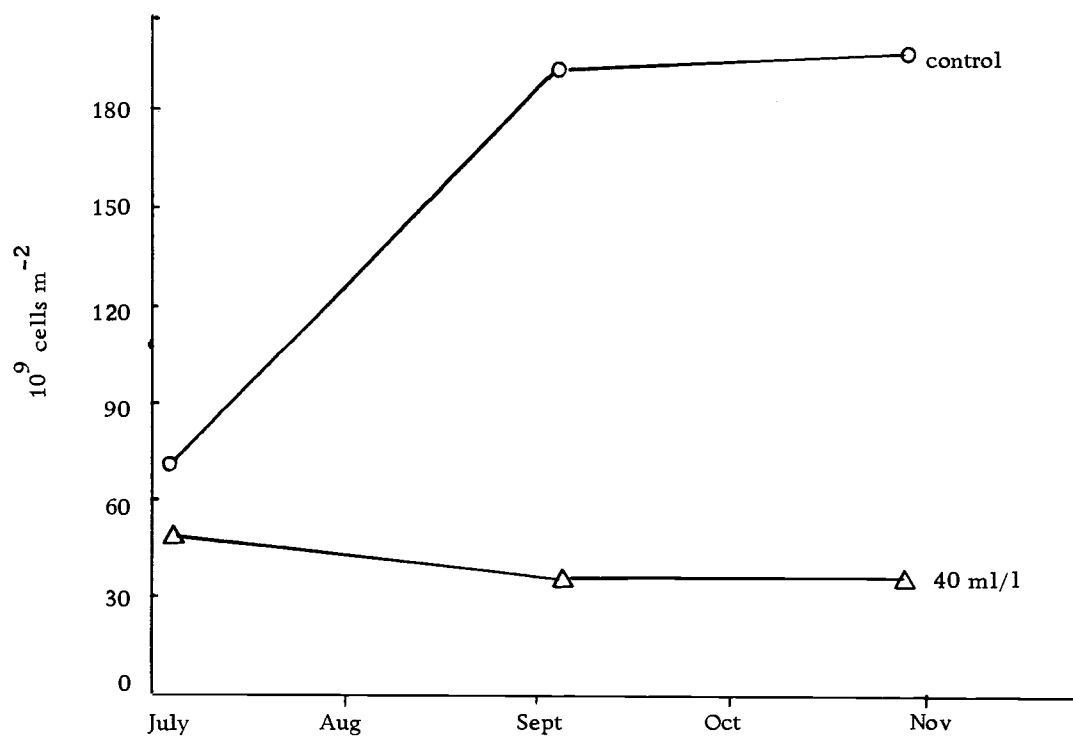


Figure 23. Comparison of total diatom cell densities in control and experimental streams as related to time and the concentration of effluent.

DISCUSSION

Community Function

The light intensity at which primary production was measured, 9,150 lux, was somewhat below the summer maximum sunlight intensity of 11,100 lux but probably above the average intensity to which the stream communities were adapted. At this intensity, the relationship between light intensity and rate of photosynthesis no longer should be linear but beginning to flatten out so that relatively small differences in light intensity should have had little effect on the rate of photosynthesis (McIntire and Phinney, 1965). Assuming that measurements made by other workers covered the same portion of the light response curve of photosynthesis some comparisons are possible.

Values obtained for gross primary production by control and experimental communities in this research varied between 76 and 438 mg of O_2 $hr^{-1} m^{-2}$ covering about the same range obtained by McIntire (1965) who lists rates of primary production of from 189 to 434 mg of O_2 $hr^{-1} m^{-2}$ (Table 7). Applying similar methods of measurement of primary production in a shallow woodland stream, Reese (1966) recorded values ranging from 52 to 203 mg of O_2 $hr^{-1} m^{-2}$ for the unenriched sections of the stream, the sections more

similar to the laboratory streams in the present research. Odum (1957) making in situ measurements using the upstream-downstream dissolved oxygen method of estimating primary production found rates in the deeper waters of Silver Springs ranging between 600 and 2900 mg of O_2 $hr^{-1} m^{-2}$.

Rates of community respiration of between 16 and 125 mg of O_2 $hr^{-1} m^{-2}$ recorded in this research were somewhat lower than the range of 104 to 171 mg of O_2 $hr^{-1} m^{-2}$ determined by McIntire and Phinney (1965). The lower rate of respiration was probably due to the lower biomass supported by the streams in the present study. An additional factor in the case of the study by McIntire and Phinney was the lower P-R ratio determined for their streams, indicating a greater degree of heterotrophy.

Respiration rates of 54.5 to 120 mg of O_2 $hr^{-1} m^{-2}$ were registered by Reese while Odum found a higher range of 117 to 208 mg of O_2 $hr^{-1} m^{-2}$.

Only at Silver Springs were P/R ratios as high as those of the communities from the laboratory streams in this experiment. The ratios varied between 1.18 and 7.0 in the laboratory streams compared to those calculated by Odum of from 2.9 to 7.0. McIntire noted P. R ratios of from 1.3 to 2.5 in his laboratory streams and Reese in the woodland streams found the highest P/R ratio to be .85, never exceeding 1.

Table 7. Rates of gross primary production, community respiration and P/R ratios of communities from experimental and control laboratory streams compared with estimates obtained from other natural and laboratory streams.

Source, date, type of stream		Gross production $\text{mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$	Community respiration $\text{mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$	P/R ratio
This research				
Raw effluent Control	High	357	76	4.27 ¹
	Low	203	30	1.85
Experimental	High	390	90	7.0
	Low	76	37	1.18
Stabilized effluent Control	High	326	90	7.0
	Low	159	12	1.3
Experimental	High	438	125	6.0
	Low	179	16	1.35
McIntire and Phinney (1965) Laboratory streams	High	434	171 ²	2.5
	Low	189	104	1.3
Reese (1966)	Control			
Woodland stream divided into four segments, two enriched with sucrose Mean values	Light	203	120	0.85
	Shade	52	54.5	0.47
	Enriched			
	Light	49	136	0.20
	Shade	44	145	0.16
Odum, Silver Springs (1956a)				
Winter 1952-3 Spring 1954		660	117	2.9
		2900	208	7.0
Odum, 1956a White River Indiana, near pollution outfall. 1934 Data adapted from Denham 1938		20	2240	0.008

¹ P/R ratios previously given have been divided by two to provide a 24 hour basis for comparison

² Appears as daily rate

Seasonal patterns were evident in comparisons between primary production by communities from control and experimental streams. Late summer rates of production on the basis of both area and unit mass of organic matter of communities from streams receiving raw effluent exceeded those of controls. During the remainder of the year, controls exceeded experimental communities in both these quantities.

Production per unit area by streams receiving stabilized effluent was greater than by controls both in summer and winter with the exception of September, 1967, immediately after the changeover from raw to stabilized effluent and February, 1968, when communities from control streams gave unusually high rates of production coincident with a high concentration of organic matter.

When primary production was expressed as rate per gram of organic matter, these aberrations from the general pattern did not appear. Production per gram of organic matter in streams receiving waste exceeded production in controls during all seasons except winter when production rates of controls were slightly higher than those of experimental streams.

The burst of productivity in control streams in February, 1968, coincided with a bloom of Fragilaria brevistriata. However, a corresponding bloom of equal magnitude of Melosira varians in experimental streams failed to offset the apparent effect of the

Fragilaria bloom. The fact that the two species were present in equal numbers and the experimental streams contained a greater total number of cells per unit area implies a high rate of primary production per cell of Fragilaria.

The ability of communities developed in streams receiving raw and stabilized effluent to exceed their respective controls in rate of production during summer months may be the result of interaction of multiple factors. Both effluents contributed mineral nutrients essential to autotrophs and heterotrophs. Both also contributed organic nutrients better utilized by heterotrophs, the raw effluent contributing much more. The presence of the organic nutrients stimulated heterotrophic activity so that in winter heterotrophs were better able to compete with autotrophs for substrate attachment and mineral nutrients. Increased solar radiation and the inorganic nutrients reversed the advantage in favor of the autotrophs in summer. The higher BOD of the raw effluent may explain the greater inhibition of primary production in streams receiving raw effluent.

In early July, 1967, the streams that had been receiving raw effluent from Mill A were supplied stabilized effluent from the same source. Mid-September sampling showed a continued superiority by control streams over experimental streams in terms of relative rates of gross primary production per unit area. This trend was accompanied

by a continuation of the drop in biomass of the experimental streams that had commenced when the intensity of the available light was reduced. By mid-November, the biomass had increased and production in experimental communities had attained record highs. Production in control communities continued at approximately the same rate from March to November.

A possible excess in production by experimental communities over control communities in September may have been prevented by the adjustment of populations to a changing environment. Populations adapted to the presence of the raw waste might have declined while new populations better adapted to conditions presented by the stabilized waste might not yet have become established.

While it is recognized that conclusions regarding the nature of factors causing fluctuations of densities of diatom populations must be approached with caution (Patrick et al., 1954; Hodgetts, 1921) the fact that population densities of some species did undergo marked changes when stabilized effluent was substituted for raw effluent cannot be ignored. The density of Synedra ulna (Figure 12) diminished to a low level while Rhoicosphenia curvata (Figure 13) and Cocconeis placentula var. euglypta (Figure 15) increased in abundance. Although the drop in abundance of Synedra ulna was a continuation of an already established trend, this taxon never regained

prominence in the experimental streams while stabilized effluent was being added, notwithstanding the fact that it underwent several fluctuations in density in the control streams.

A statistically significant difference between mean rates of oxygen consumption by experimental and control communities occurred during the period of treatment with stabilized effluent both on the basis of consumption per unit area and per unit mass of organic matter. Unlike production which sometimes increased several times between successive observations, rates of oxygen consumption per unit area or per unit mass of organic matter formed smoother curves, changing by more or less equal increments from one observation to the next.

Seasonal patterns of rates of oxygen consumption per unit area of communities tended to reflect the rates of gross primary production. When communities from control streams displayed higher rates of production than those from experimental streams, they also tended to display higher rates of oxygen consumption. When rates of production were high, rates of oxygen consumption were high. Conversely, when rates of production were low, oxygen consumption was low.

Exceptions to the general pattern occurred in June, 1967 when communities from streams receiving raw effluent had lower rates of production per unit area but higher rates of oxygen consumption than did their controls, perhaps influenced by higher water temperatures

in the spring; November, 1967, when communities from streams receiving stabilized effluent exhibited higher rates of production and lower rates of oxygen consumption per unit area and per unit mass of organic matter than did controls, and in February, 1968 when communities from streams receiving stabilized effluent exhibited lower rates of production and higher rates of oxygen consumption than did controls.

Reversals of relationships between production and consumption of oxygen by experimental and control communities correlated best with chlorophyll a assimilation number. A higher assimilation number appeared to indicate a higher P/R ratio. The communities with higher rates of production and lower rates of respiration also contained higher concentrations of chlorophyll a per unit area. Two of three cases in which reversals occurred coincided with higher concentrations of chlorophyll a per unit mass of organic matter in the more productive communities while in the third, the community with the lower rate of production contained only slightly less chlorophyll per unit mass of organic matter. Organic matter per unit area was less in June but greater in November and February in the communities with higher rates of production.

No consistent relationship between densities of diatom populations and the relative rates of production and oxygen consumption were apparent.

The close parallel between rates of production and rates of respiration implies an important role for primary producers as consumers of oxygen in these streams. Rates of respiration by heterotrophic organisms would vary independently of rates of production, hence communities comprising a large heterotrophic component would not evidence such a close parallel between rates of production and respiration.

Comparisons of P/R ratios between experimental and control streams were calculated by dividing hourly rates of gross primary production by hourly rates of oxygen consumption. Twenty-four hour P/R ratios were entered in Table 7 to facilitate comparisons with values obtained by other workers.

The laboratory streams resembled natural streams near their headwaters in that they had high P/R ratios and a predominance of primary producers (Odum, 1956). The ratios never fell below one and in one occasion, December, 1967, reached 14 in experimental streams and 12 in control streams. These high ratios were due to extremely low rates of respiration coincident with the lowest water temperatures and lowest biomasses of the year. This observation agrees with the findings of Phinney and McIntire (1965) that at light intensities below saturation, oxygen consumption by periphyton communities was affected by temperature but photosynthesis was not.

Communities from streams receiving raw effluent always recorded lower P/R ratios than control streams indicating a higher degree of heterotrophic activity. Introduction of stabilized effluent produced no consistent effect, experimental communities sometimes yielding higher and sometimes lower ratios than controls. However, the average of the P/R ratios of streams receiving stabilized effluent was slightly higher than that of the controls, perhaps due to enhancement of primary production.

In September and November, 1967, P/R ratios of experimental communities exceeded those of controls by the greatest margin correlating with the higher concentrations of chlorophyll a per gram of organic biomass in those streams.

Students' t-test showed a significantly greater mean concentration of organic matter in experimental streams during treatment with raw effluent. All three observations and five of seven observations during treatment with stabilized effluent showed higher biomass in experimental streams.

One of the observations in which control streams yielded higher concentrations of organic matter per unit area occurred in the summer of 1967, subsequent to switching from raw to treated effluent.

Reaching a maximum in March, 1967, organic matter per

unit area in the streams followed parallel courses until July when the organic matter per unit area of the experimental streams fell below that of the controls, the decline from peak levels of organic matter per unit area coinciding with installation of the saron shading.

Over the two year period, the control streams experienced three relatively major peaks and one smaller peak concentrations of organic matter while experimental streams peaked only twice, once each during the successive late winter-early spring periods. Conclusions as to a causal relationship between these differences and Kraft mill enrichment would require at least another year's sampling.

The dominant diatom during the application of raw effluent was Synedra ulna, the population density of which closely paralleled organic matter per unit area in control and experimental streams. Some significance may be attached to the fact that production per unit area of organic matter varied inversely with the population density of this diatom perhaps implying a degree of facultative heterotrophism for this organism.

Mean values for chlorophyll a per square meter were higher in experimental streams receiving raw effluent than in controls. However, the difference was not great enough to meet Students' t-test criteria of significance. During treatment with stabilized effluent, control and experimental streams supported almost identical concentrations of chlorophyll a per unit area.

Streams receiving raw effluent supported more chlorophyll a m^{-2} than did controls in January and March, 1967. In June, the concentration of chlorophyll a m^{-2} in the controls exceeded slightly the level in the experimental streams. It is possible that the reduced light level in the presence of the effluent was responsible for the greater depression of chlorophyll a m^{-2} in the experimental streams, however on the basis of the limited evidence at hand, such a relationship would be difficult to substantiate.

Control streams maintained a higher level of chlorophyll a m^{-2} than did streams receiving stabilized effluent until November when the experimental streams regained superiority and retained it through December. In January, chlorophyll a in the control streams slightly exceeded that in the experimental streams. In March, the record high was reached for the two year period of treatment with effluent from mill A. The chlorophyll a concentration then dropped rapidly so that from April through July, the experimental streams contained a higher concentration of chlorophyll a than did the controls.

Some tentative correlations may be made between peak densities of some diatom populations and peak concentration of chlorophyll a per gram of organic matter. The November chlorophyll peak in control streams coincides with a peak in total population density (Figures 7 and 15).

Table 8. Comparison of the concentrations of chlorophyll a as related to unit area by various investigators as tabulated by Moss (1967a).

Source		Date	Chlorophyll <u>a</u> mg m ⁻²
Present study		1969	
Raw effluent			
Control	High		374
	Low		100
Experimental	High		249
	Low		92
Stabilized effluent			
Control	High		776
	Low		252
Experimental	High		599
	Low		173
Brock and Brock, hot springs USA and Iceland at optimum temperature		1966	800
Margalef, stream, Aigues Tortes, Northern Spain		July 1959	260
McConnell and Sigler, calcereous mountain river, (Logan) USA		Yearly averages at 2 stns	300- 1200
McIntire, experimental laboratory streams		1966	233 (Oedogonium) 381 (Diatoms)

The same peak in the experimental streams coincides with high population density of Rhoicosphenia curvata (Figure 14). In February and March, blooms of Melosira varians occurred in both experimental and control streams but of greater magnitude in the former (Figure 16) accompanied by a bloom of Synedra ulna (Figure 12) and Fragilaria brevistriata (Figure 18) in the control streams.

During treatment with waste from the stabilization pond of mill B chlorophyll a approached Odum's (1959) postulated maximum of one g chlorophyll a m⁻² and reached a concentration of 890 mg in October, coincident with an enormous bloom of the diatom Fragilaria brevistriata, which did not occur at that time in the experimental streams. Several other diatoms contributed to this high density of chlorophyll a. Those whose mean density in the control streams exceeded their mean density in experimental streams for the three month period are Navicula (sp.), Synedra ulna, F. brevistriata, and Melosira varians.

During the first year of the experiment, under exposure to sunlight subject only to diffusion through the fiberglass roof of the laboratory, assimilation numbers (mg O₂ hr⁻¹ mg⁻¹ of chlorophyll a) for the laboratory stream communities ranged from a minimum of 0.45 in January to 3.21 in June. These values were in the range cited by Odum (1959) of between 0.5 and 6.3 mg oxygen hr⁻¹ mg⁻¹ for a variety of terrestrial and aquatic ecosystem.

The second year after shading was added, light intensities ranged seasonally from 1,290 to 12,900 lux. Assimilation numbers were lower and varied over a narrower range, between 0.56 and $0.98 \text{ mg O}_2 \text{ hr}^{-1} \text{ mg}^{-1}$. This behavior is consistent with that cited by Odum (1956a) indicating that assimilation numbers tend to remain more constant in spite of variations in light intensity in shade adapted than in sun adapted communities.

Assimilation numbers of communities in control streams were higher in all three measurements taken during the time when experimental streams were receiving raw effluent. During treatment with stabilized waste, half the observations showed higher assimilation numbers in controls and half higher in the experimental streams.

A seasonal pattern of relationships of the assimilation numbers was discernible. In the fall of 1967, in the presence of raw effluent assimilation numbers of experimental streams exceeded those of controls but when stabilized effluent was substituted for raw effluent, they dropped below the level of the controls. A continuation of a downward trend that began with the installation of shading the previous March, the decrease in assimilation number was accompanied by a downward trend in organic matter per unit area while chlorophyll \underline{a} m^{-2} remained relatively constant in controls

and increased in experimental streams.

By November, the assimilation number in the experimental streams was slightly higher than in the controls but the two winter readings, December and February, placed the controls somewhat higher once more. By spring, experimental streams again were registering higher assimilation numbers. These readings appear to represent enhancement of assimilation number by stabilized effluent during periods of greatest light intensity similar to that noted for production per gram of organic matter.

The decrease in biomass in the experimental streams when shading was installed in March, 1967, may have been the result of these communities being less able than the controls to withstand stress. When the light level was suddenly lowered, disruption was greater producing a more dramatic and extended effect. The decrease may have occurred as senescent cells and those primary producers less able to adapt to low light intensity were swept away. With the loss of marginal producers, the assimilation number representing the remaining more efficient producers would be expected to increase.

The replacement of raw effluent by stabilized effluent created conditions favorable to the development of new populations, modifying the communities and reducing the assimilation number but

increasing chlorophyll a per gram of organic matter as the amount of organic matter continued to decline. Perhaps some populations of facultative heterotrophs low in chlorophyll and favored by raw waste disappeared. The inverse correlation between population density of Synedra ulna and chlorophyll a per gram of organic matter further suggests that it may be a facultative heterotroph contributing to the effect described above.

The relatively large drop in biomass of experimental stream communities following shading failed to produce a corresponding reduction in production and respiration per unit area resulting in increased production, respiration, and chlorophyll a per gram of biomass, an increase in chlorophyll a per unit area and a reduced assimilation number.

Dramatic changes in structure in both control and experimental stream communities reflected these changes in function. Stigeoclonium subsecundum so abundant up to that time completely disappeared, never to reappear. Oedogonium (sp.) only rarely observed prior to shading became abundant albeit never achieving the ascendancy of Stigeoclonium. Diatoms, especially Synedra ulna completely dominated the communities in both control and experimental streams. These new communities, shade adapted and burdened with fewer senescent cells were perhaps able to cycle

energy at a greater rate per unit biomass than were their predecessors thereby maintaining the former rate of production per unit area.

Although the presence or absence counts show Oedogonium to have appeared in a greater number of fields than did Stigeoclonium, the filaments of Oedogonium have a much greater tendency to fragment during mixing in the blender and this is believed responsible for its apparent greater abundance.

Enhancement of respiration by the effluent demonstrated in the P-R chamber may be attributed either to available mineral nutrients in the effluent or to residual BOD serving as respiratory substrate for heterotrophic metabolism (Table 6). The latter seems unlikely since the BOD of the added effluent never exceeded 14 ppm and was diluted 25 fold yielding a BOD only slightly higher than that present in the streams receiving effluent.

Enhancement of respiration more likely was due to the presence of some substance metabolically active when present in low concentrations. The nitrate and phosphate added to the effluent during the stabilization process may have been the factors responsible. In the natural stream water, nitrate concentration ranged between 0.05 and 0.2 mg per liter and phosphate concentration between 0.04 and 0.10 mg per liter.

Nitrogen and phosphate were added to the effluent at rates providing concentrations when diluted equal to 0.48 mg per liter for phosphate and 3.6 mg per liter for nitrate. These values exceed concentrations listed by Fogg (1965) for a eutrophic lake of 0.012 mg per liter for phosphates and 0.05 to 0.22 mg per liter for nitrate.

Community Structure

Total population densities of diatoms in the experimental streams generally followed no consistent pattern relative to densities in the control streams during the two year period of the research (Figure 17). Neither did densities correlate well with any of the functional parameters.

Only Synedra ulna appeared to become more abundant in the presence of raw effluent (Figure 12). Melosira varians (Figure 14), Cocconeis placentula (Figure 16) and Rhoicosphenia curvata (Figure 13) showed reduced population densities in experimental streams during the period of application of raw effluent but appeared to be favored by stabilized waste. However, the population density of Melosira varians was lower in the presence of waste from the stabilization pond of mill B.

Fragilaria brevistriata (Figure 15) first appeared in control streams in September, 1967 reaching a peak abundance in February, 1968. It made only two brief appearances in the experimental streams during this time becoming as abundant as in the control streams on a single occasion, May, 1968.

Effluent from the stabilization pond of mill B appeared to inhibit Fragilaria brevistriata. The population densities of the organism on September 3, 1968 varied inversely with effluent concentration except in the stream receiving 5 ml of effluent per liter of water.

Synedra ulna, so abundant in the presence of raw effluent was less abundant in the presence of the stabilized effluents from both mills. Population densities on September 3 also varied inversely with effluent concentration.

Cocconeis placentula, var. euglypta and Rhoicosphenia curvata both seemingly inhibited by raw effluent appeared to be stimulated by stabilized effluent from mill A. When effluent from the stabilization pond of mill B was applied, the population density of R. curvata increased even more.

When control and experimental streams are compared on the basis of the densities of the total diatom population (Figure 17) no

obvious pattern emerges except for the period of treatment with waste from mill B. During this period, control streams supported denser populations of diatoms than did experimental streams. It was during this time that F. brevistriata and M. varians were abundant and it is to these two species that a large measure of this greater population density in control streams may be attributed.

Two peak population densities occurred during treatment with effluent from mill A, the first in January, 1967 in experimental streams receiving raw waste and the second in controls during November, 1967 when stabilized effluent was being applied. Significantly, both these peaks coincide with blooms of Synedra ulna. Synedra ulna was more abundant in streams receiving raw effluent and less abundant in streams receiving stabilized effluent than in the respective control streams.

Table 9. Comparison of effects of raw and stabilized waste on function of communities from laboratory streams.

Possible Effect	Level of Significance	Heterotrophic	Autotrophic	Neither
TREATMENT WITH RAW EFFLUENT				
Reduced primary production m^{-2}	< 0.10	X		
Reduced primary production g^{-1}	< 0.01	X		
Increased respiration m^{-2}	< 0.20			X
Reduced respiration g^{-1}	< 0.10			X
Reduced P/R ratio	< 0.05	X		
Increased organic matter m^{-2}	< 0.10			X
Increased chlorophyll a m^{-2}	< 0.15		X	
Reduced chlorophyll a g^{-1}	< 0.20	X		
Reduced assimilation number	< 0.10	X		
TREATMENT WITH STABILIZED EFFLUENT				
Increased primary production m^{-2}	< 0.45		X	
Increased primary production g^{-1}	< 0.025		X	
Increased respiration m^{-2}	< 0.10			X
Increased respiration g^{-1}	< 0.05			X
Increased P/R ratio	< 0.45		X	
Reduced organic matter m^{-2}	< 0.25			X
Reduced chlorophyll a m^{-2}	< 1			X
Increased chlorophyll a g^{-1}	< 0.10		X	
Reduced assimilation number	< 1			X

SUMMARY AND CONCLUSIONS

A number of effects on the function of periphyton communities can be attributed to the presence of both raw and stabilized Kraft mill effluent. The fact that the experiments were carried out in succeeding years of greatly dissimilar weather renders direct comparisons between the effects of the two effluents impossible. Indirect comparisons may be made however, by comparing experimental and control streams during the first year when raw effluent was applied with those during the second when stabilized effluents were used. The raw effluent appeared to enhance those properties associated with heterotrophy while the latter favored autotrophy (Table 9). Although some of the contrasts listed failed to meet the 0.10 level of significance, it seems significant that with only one exception, the differences between experimental and control streams in all the relevant parameters follow this trend. The single exception, chlorophyll a per unit area showed a higher mean value for streams receiving raw waste and slightly lower mean value for streams receiving stabilized waste than for control streams. Comparisons between control and experimental streams strictly on the basis of mean values tend to conceal seasonal patterns. These patterns are more apparent when values for each sampling period are presented in graphic form.

Stabilized effluents produced nearly as many significant differences in community function and structure as did raw effluent. Differences between means for estimates of four functional parameters were significant at the 0.10 level in the presence of stabilized effluent and six in the presence of raw effluent. Population densities of six diatoms differed significantly between experimental and control streams while stabilized effluent was applied while only four differed significantly during application of raw effluent. The importance of these differences in numbers of significant effects is dimmed somewhat by the fact that so few observations were made during the first year.

That stabilized effluent rich in nitrates and phosphates added during stabilization and the products of bacterial action may equal raw effluent in its effect on a stream is of concern. It must be considered in evaluation of treatment procedures intended to minimize ecological disturbance to a stream receiving Kraft mill wastes.

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APPENDIX

Appendix Table 1. Rates of gross primary production as measured in P-R chamber under 9, 150 lux illumination.
(mg O₂ hr⁻¹ m⁻²)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Sept. 7, 1966	357	390		
Jan. 3, 1967	203	76		
March 15, 1967	335	269		
June 6, 1967	321	217		
Sept. 15, 1967			303	272
Nov. 20, 1967			310	438
Dec. 20, 1967			159	179
Feb. 19, 1968			326	243
April 30, 1968			231	304
July 5, 1968			215	334*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 2. Rates of gross primary production as measured in P-R chamber under 9, 150 lux illumination.
(mg O₂ hr⁻¹ g⁻¹ ash-free dry weight)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Jan. 3, 1967	5.95	1.49		
March 15, 1967	2.68	1.69		
June 6, 1967	5.79	2.55		
Sept. 15, 1967			4.2	14.0
Nov. 20, 1967			11.2	15.0
Dec. 20, 1967			7.13	5.33
Feb. 19, 1968			5.50	5.10
April 30, 1968			6.40	7.70
July 5, 1968			8.60	11.8*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 3. Rates of oxygen consumption as measured in P-R chamber. ($\text{mg O}_2 \text{ hr}^{-1} \text{ m}^{-2}$)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Sept. 7, 1966	30	47		
Jan. 3, 1967	40	37		
March 15, 1967	76	63		
June 6, 1967	63	93		
Sept. 15, 1967			73	59
Nov. 20, 1967			58	39
Dec. 20, 1967			12	16
Feb. 19, 1968			54	84
April 30, 1968			90	113
July 5, 1968			70	125*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 4. Rates of oxygen consumption as measured in P-R chamber. ($\text{mg O}_2 \text{ hr}^{-1} \text{ g}^{-1}$ ash-free dry weight)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/l	Control	15 ml/l
Jan. 3, 1967	1.17	0.49		
March 15, 1967	0.61	0.40		
June 6, 1967	1.14	1.10		
Sept. 15, 1967			1.00	3.10
Nov. 20, 1967			2.10	1.33
Dec. 20, 1967			0.53	0.47
Feb. 19, 1968			0.92	1.77
April 30, 1968			2.49	2.85
July 5, 1968			2.80	4.46*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 5. Ratio of photosynthesis to respiration (P/R ratio).

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/l	Control	15 ml/l
Sept. 7, 1966	8.54	7.30		
Jan. 3, 1967	5.08	3.05		
March 15, 1967	4.41	4.27		
June 6, 1967	5.09	2.36		
Sept. 15, 1967			4.18	4.67
Nov. 20, 1967			5.35	11.4
Dec. 20, 1967			14	12
Feb. 19, 1968			6.0	2.9
April 30, 1968			2.6	2.7
July 5, 1968			3.1	2.7*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 6. Ash-free dry weight per unit area. (g m^{-2})

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Jan. 3, 1967	34	75		
March 15, 1967	125	159		
June 6, 1967	55	85		
Sept. 15, 1967			72	19
Nov. 20, 1967			28	29
Dec. 20, 1967			22	34
Feb. 19, 1968			59	47
March 20, 1968			29	44
April 30, 1968			36	40
July 5, 1968			25	28*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 7. Concentration of chlorophyll a (mg m⁻²)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Jan. 3, 1967	174	249		
March 15, 1967	124	150		
June 6, 1967	100	092		
Sept. 15, 1967			310	173
Nov. 20, 1967			412	541
Dec. 20, 1967			252	414
Feb. 19, 1968			478	455
March 20, 1968			776	599
April 30, 1968			409	398
July 5, 1968			325	371*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 8. Concentration of chlorophyll a (mg g⁻¹ ash-free dry weight)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Jan. 3, 1967	5.10	3.30		
March 15, 1967	0.99	0.94		
June 6, 1967	1.81	1.08		
Sept. 15, 1967			4.30	9.1
Nov. 20, 1967			14.9	18.7
Dec. 20, 1967			11.3	12.3
Feb. 19, 1968			8.1	9.6
March 20, 1968			26.4	13.6
April 30, 1968			11.3	10.2
July 5, 1968			12.9	13.0*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 9. Rate of oxygen production per mg of chlorophyll a ($\text{O}_2 \text{ hr}^{-1} \text{ mg}^{-1}$ chlorophyll a) (Assimilation number)

Date	Raw effluent		Stabilized effluent	
	Control	15 ml/1	Control	15 ml/1
Jan. 3, 1967	1.16	0.45		
March 15, 1967	2.7	1.8		
June 6, 1967	3.21	2.4		
Sept. 15, 1967			0.98	0.63
Nov. 20, 1967			0.75	0.81
Dec. 20, 1967			0.63	0.43
Feb. 19, 1968			0.68	0.53
April 30, 1968			0.56	0.76
July 5, 1968			0.66	0.90*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 10. Densities of populations of abundant diatoms during exposure to effluent from mill A (10^9 diatoms m^{-2}).

Date	Navicula <u>sp.</u>		Nitschia <u>sp.</u>		Gomphonema <u>sp.</u>	
	Control	15 ml/1	Control	15 ml/1	Control	15 ml/1
RAW EFFLUENT						
Jan. 3, 1967	1.45	3.69	6.50	49.2	7.95	29.6
March 15, 1967	0.715	2.89	9.38	18.7	5.05	1.45
June 6, 1967	0.715	0.715	10.13	2.90	2.88	3.60
STABILIZED EFFLUENT						
Sept. 15, 1967	0	7.15	5.75	2.16	2.89	2.89
Nov. 20, 1967	7.23	0	47.7	0.715	31.8	0
Dec. 20, 1967	0	0.715	0.715	1.43	0	0.715
Feb. 19, 1968	1.43	0.715	1.43	2.86	1.43	2.17
March 20, 1968	0.715	0	2.90	3.46	0	1.45
April 30, 1968	0	2.17	0.715	12.3	0.715	1.43
July 5, 1968	7.95	1.43*	18.1	7.92*	4.33	2.17*

Appendix Table 10. Continued

Date	<u>Achnanthes lanceolata</u>		<u>Synedra ulna</u>		<u>Rhoicosphenia curvata</u>	
	Control	15 ml/1	Control	15 ml/1	Control	15 ml/1
RAW EFFLUENT						
Jan. 3, 1967	5.80	9.58	0.234	4.58	7.23	0.965
March 15, 1967	3.90	8.30	0.481	5.97	1.09	0
June 6, 1967	5.55	3.39	0.440	1.03	20.1	0.234
STABILIZED EFFLUENT						
Sept. 15, 1967	3.40	2.52	0.274	0.138	1.06	5.51
Nov. 20, 1967	7.44	1.38	3.39	0.0552	0.428	8.11
Dec. 20, 1967	0.621	2.87	0.0552	0.0138	1.24	7.89
Feb. 19, 1968	1.93	2.30	1.64	0.0276	3.23	6.78
March 30, 1968	1.46	4.94	1.13	0.138	1.57	3.59
April 30, 1968	0.773	3.93	0.041	0.360	1.19	4.57
July 5, 1968	3.78	2.55*	0.304	0.179*	3.66	4.70*

Appendix Table 10. Continued

Date	<u>Fragilaria brevistriata</u>		<u>Cocconeis placentula</u> var. <u>euglypta</u>		<u>Achnanthes minutissima</u>	
	Control	15 ml/1	Control	15 ml/1	Control	15 ml/1
RAW EFFLUENT						
Jan. 3, 1967	0	0	2.52	0.51	12.4	12.5
March 15, 1967	0	0	0.88	0.51	9.70	5.40
June 6, 1967	0	0	2.88	0.51	6.04	1.76
STABILIZED EFFLUENT						
Sept. 15, 1967	0.796	0	1.52	3.66	3.87	1.89
Nov. 20, 1967	0	0	1.64	3.41	21.3	0.883
Dec. 20, 1967	3.62	2.90	1.31	3.33	0.469	2.15
Feb. 19, 1968	6.51	0	3.16	3.22	1.61	2.24
March 20, 1968	0.29	0	1.61	2.08	1.70	2.47
April 30, 1968	1.43	1.43	0.773	3.09	0.54	3.09
July 5, 1968	34.0	23.1*	2.44	2.00*	4.00	1.85*

Appendix 10. Continued

Date	<u>Melosira varians</u>		Total	
	Control	15 ml/l	Control	15 ml/l
RAW EFFLUENT				
Jan. 3, 1967	0.327	0.0276	44.4	110
March 15, 1967	0.687	0.426	31.8	43.6
June 6, 1967	3.32	3.17	52.0	14.4
STABILIZED EFFLUENT				
Sept. 15, 1967	0.910	0.566	20.6	20.1
Nov. 20, 1967	0.138	1.04	121	15.6
Dec. 20, 1967	0.497	0.759	8.52	22.8
Feb. 19, 1968	0.620	6.91	23.0	27.3
March 30, 1968	4.01	4.79	15.4	23.1
April 30, 1968	1.90	5.45	8.10	37.8
July 5, 1968	3.79	2.83*	82.2	48.1*

*Effluent added at the rate of 40 ml per liter of water

Appendix Table 11. Densities of populations of abundant diatoms during exposure to effluent from stabilization pond of mill B. (10^9 diatoms m^{-2}).

Date	Control	5 ml/l	10 ml/l	20 ml/l	40 ml/l
<u>Navicula</u> (sp)					
Sept. 3, 1968	2.17	0	0.715	0.715	0.715
Oct. 28, 1968	1.89				1.38
<u>Nitzschia</u> (sp)					
Sept. 3, 1968	4.33	0.715	5.78	6.51	2.17
Oct. 28, 1968	1.01				2.90
<u>Fragilaria brevistriata</u>					
Sept. 3, 1968	175	10.1	77.4	34.0	20.2
Oct. 28, 1968	184				18.8
<u>Gomphonema</u> (sp)					
Sept. 3, 1968	0.715	1.43	0	1.43	0
Oct. 28, 1968	0.76				0.635
<u>Cocconeis placentula</u> var. <u>euglypta</u>					
Sept. 3, 1968	1.31	1.43	3.63	2.17	0.925
Oct. 28, 1968	1.38				1.89
<u>Achnanthes minutissima</u>					
Sept. 3, 1968	1.53	1.08	1.93	2.93	2.54
Oct. 28, 1968	1.52				3.02

Appendix Table 11. Continued

Date	Control	5 ml/l	10 ml/l	20 ml/l	40 ml/l
<u>Achnanthes lanceolata</u>					
Sept. 3, 1968	2.77	1.70	1.78	4.86	2.93
Oct. 28, 1968	1.77				0.76
<u>Synedra ulna</u>					
Sept. 3, 1968	0.730	0.0138	0.19	0.041	0.041
Oct. 28, 1968	0.248				0.124
<u>Rhoicosphenia curvata</u>					
Sept. 3, 1968	0.59	1.41	1.44	4.06	3.06
Oct. 28, 1968	0				11.1
<u>Melosira varians</u>					
Sept. 3, 1968	4.21	2.73	5.31	3.93	2.32
Oct. 28, 1968	4.28				0.88
Total Density					
Sept. 3, 1968	193	20.7	98.2	60.7	34.9
Oct. 28, 1968	198				38.2

Appendix Table 12. Total dissolved solids (TDS), volatile solid, chemical oxygen demand (COD), and biochemical oxygen demand (BOD) of Kraft mill effluent applied to experimental streams (Mill A).

Date	TDS ppm	Volatile solids ppm	COD (mg/liter)	BOD (mg/liter)
January 25, 1967	582	222	462	171
February 8	502	185	528	206
February 15	787	290	746	267
March 1	598	230	575	230
March 8	628	215	536	200
March 15	684	235	600	195
March 29	716	277	753	260
April 11	591	217	542	180
April 19	764	275	750	235
April 26	686	247	670	290
May 3	570	197	593	166
May 10	547	171	526	222
May 17	693	234	575	221
May 24	654	209	624	257
June 1	-	-	-	-
June 8	-	-	-	-
June 15	-	-	-	-
June 22	-	-	-	-
June 29	625	191	554	230
July 8	875	324	748	260

Appendix Table 13. Biochemical and chemical oxygen demand of Kraft mill effluent before and after stabilization (Mill A).

Date	Before treatment		After treatment	
	BOD	COD	BOD	COD
July 12, 1968	183	512	6	--
July 17	135	431	5	--
July 26	170	--	8	--
August 2	156	422	6	--
August 9	156	439	5	--
August 16	135	481	8	--
August 23	188	--	2	--
August 30	138	--	1	--
September 6	192	--	7	--
September 13	143	--	3	--
September 20	184	--	0	71
September 27	191	594	0	122
October 11	174	481	--	--
October 18	201	412	5	--
October 25	252	442	3	78
November 1	214	436	3	70
November 8	195	473	2	75
November 22	262	716	10	--
December 6	214	587	72	232
December 13	--	645	35	332
December 20	273	538	31	240
December 28	239	609	53	165

Appendix Table 13. Continued

Date	Before treatment		After treatment	
	BOD	COD	BOD	COD
January 18, 1968	222	325	30	210
January 25	230	555	42	226
March 1	279	720	18	203
March 8	195	265	24	184
March 15	256	575	14	158
March 22	279	632	24	200
March 29	214	440	8	180
March 8	200	424	8	164
March 15	243	664	26	254
March 21	256	545	46	322
March 28	213	752	43	358
April 4	317	1008	55	396
April 12	224	675	53	349
April 18	279	719	90	365
April 26	245	674	7	195
May 16	236	608	15	226
May 23	246	731	15	227
May 29	220	644	15	--
June 7	219	679	13	--
June 13	240	891	7	--
June 20	268	--	13	--

Appendix Table 14. Biochemical and chemical oxygen demand of Kraft mill effluent from stabilization pond of Mill B.

Date	BOD ₅	COD
July 3	72	183
July 11	43	252
July 18	44	224
July 25	24	123
August 1	20	90
August 8	9	63
August 15	35	67
August 22	--	--
August 29	--	--
September 5	19	220
September 12	37	131
September 19	26	116
September 26	28	66
October 4	34	145
October 8	40	215
October 16	69	193
October 22	52	121

Appendix Table 15. Mean quality of water in laboratory streams for years 1961, 1962, and 1963.¹

Property	March	May	August ²	November ²
Specific conductance (micromhos at 25°C.)	102	165	206	165
pH	7.5	7.9	8.0	7.6
Color	18	5	10	20
Dissolved solids mg/l	86	117	152	120
Hardness mg/l as CaCO ₃	42	70	92	60
Silica (SiO ₂) as mg/l	22	33	90	32
Iron (Fe) mg/l	39	0.11	0.34	.18
Calcium (Ca) mg/l	10	18	24	18
Magnesium (Mg) mg/l	3.7	6.1	8.0	6.2
Sodium (Na) mg/l	4.6	7.3	9.2	8.2
Potassium (K) mg/l	0.3	0.3	0.8	0.7
Bicarbonate (HCO ₃) mg/l	56	97	126	93
Carbonate (CO ₃) mg/l	0	0	0	0
Sulfate (SO ₄) mg/l	1.0	0.4	1.1	1.4
Chloride (Cl) mg/l	4.5	4.5	4.8	6
Fluoride (F) mg/l	0.1	0.1	0.1	0.1
Nitrate (NO ₃) mg/l	3.0	0.2	0.2	.5
Phosphate (PO ₃) mg/l	0.04	0.06	0.13	.17

¹ Analyses made under the supervision of L. B. Laird, District Chemist, U. S. Geological Survey, Portland, Oregon

² For years 1961 and 1962 only

Appendix Table 16. List of algal taxa observed in laboratory streams from October 1966 through October 1968.

Chrysophyta

Bacillariophyceae

Achnanthes exigua Krasske
Achnanthes lanceolata (Breb.) Grun
Achnanthes linearis (W. Sm.) Grun
Achnanthes minutissima Kütz.
Cocconeis disculus (Schum.) Cl. var. *disculus*
Cocconeis placentula var. *euglypta* (Ehr.) Cl.
Clyclotella menegheniana Kütz.
Cymbella mexicana Ehr.
Cymbella sinuata Gregory
Cymbella tumida (Breb.) V. Heurck
Diatoma vulgare var. *brev.* Grun
Eunotia maior (W. Sm.) Rabh. var. *maior*
Epithemia turgida (Ehr.) Kütz.
Fragilaria brevistriata var. *capitata* Heriba
Fragilaria capucina Pesm. var. *capucina*
Fragilaria Croteninsis Kitton var. *crotonensis*
Fragilaria vaucheria (Kütz.) Peters var. *vaucheria*
Fragilaria virescens Ralfs
Frustulia rhomboides (Ehr.) DeT. var. *rhomboides*
Frustulia vulgaris (Thwaites) DeT.
Gomphoneis herculeana (Ehr.) Cleve
Gomphonema acuminatum var. *coronata* (Ehr.) Rabh.
Gomphonema angustatum (Kütz.) Rabh.
Gomphonema parvulum (Kütz.) Rabh.
Hantzschia amphioxys fo. *capitata* Hust.
Melosira varians Ag.
Meridion circulare (Grev.) Ag.
Navicula cryptocephala Kütz.
Navicula radiosa Kütz.
Navicula rhyncocephala Kütz.
Navicula seminulum Grun var. *seminulum*
Nitzschia amphibia Grun

Nitzschia columbiana Sov.
Nitzschia dissipata (Kütz.) Grun
Nitzschia fonticola Grun
Nitzschia frustulum var. *perminuta* (Rabh.) Grun
Nitzschia frustulum var. *perpusilla* (Rabh.) Grun
Nitzschia kutzingiana Hilse
Nitzschia linearis (Ag.) W. Smith
Nitzschia oregona Sov.
Nitzschia palea (Kütz.) W. Smith
Nitzschia sublinearis Hust.
Pinnularia acuminata var. *interrupta* (Cl.) Patr.
Pinnularia brunii var. *amphicephala* (A. Mayer) Husted
Pinnularia gentilis (Donk.) Cl. var. *gentilis*
Rhoicosphenia curvata (Kutz.) Grun
Surirella angustata Kutz.
Surirella elegans Ehr.
Surirella ovata Kütz.
Synedra acus Kütz.
Synedra fasciculata var. *truncata* (Grev.) Patr.
Synedra incisa var. *incisa* Boyer
Synedra socia Wallace
Synedra rumpens (Kütz.)
Synedra ulna (Nitz.) Ehr.

Chlorophyta

Oedogonium sp.
Stigeoclonium subsecundum Kütz.
Ulothrix variabilis Kütz.

Cyanophyta

Anabaena variabilis Kütz.
Nostoc microscopicum Carmichael
Phormidium retzii (Ag.) Gom.

Rhodophyta

Batrachospermum sp.

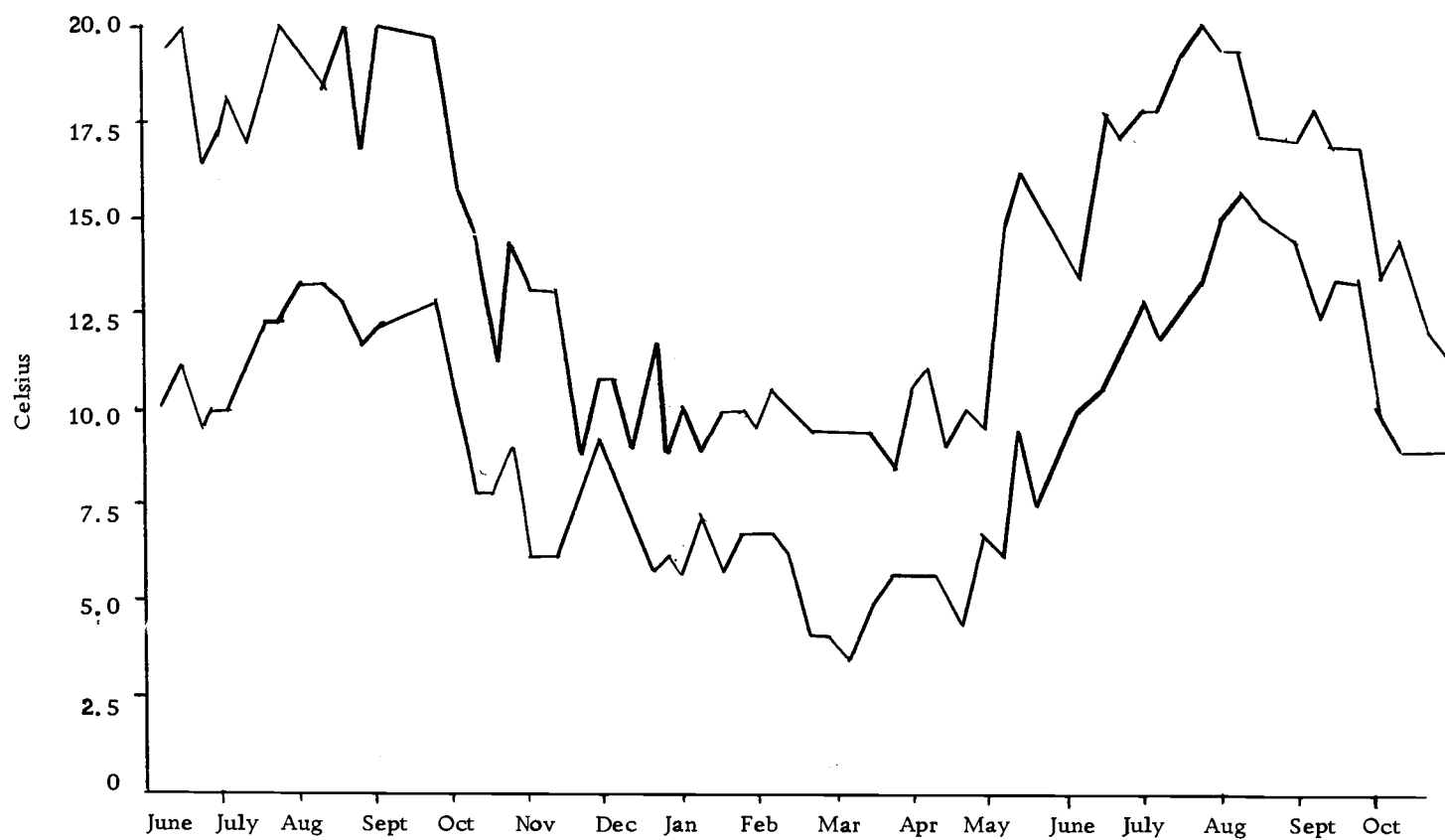


Figure 24. Mean weekly maximum and minimum water temperatures from June 7, 1966 to October 31, 1968.



Figure 24. (Continued) November 1, 1968 to October 25, 1969.