EFFECTS OF PULP AND PAPER MILL EFFLUENT ON STREAM PRIMARY PRODUCTIVITY IN THE LOWER SULPHUR RIVER, TEXAS

THESIS

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By

Terrence Marvin Davis, B.S.

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Responses of periphyton and phytoplankton productivity in the lower Sulphur River (Texas-Arkansas) to bleach-kraft mill effluent (BKME) were monitored using in situ ^{14}C incubation. Carbon assimilation rates measured downstream of mill discharge were substantially reduced from upstream levels. Periphyton and phytoplankton chlorophyll a concentrations remained relatively unchanged by the presence of BKME. Periphyton ash-free dry weight increased near the mill outfall, but decreased further downstream. Calculated productivity efficiencies (productivity:biomass) varied with variations in ^{14}C rates.

A laboratory bioassay was designed to determine the effect of BKME light-attenuation on photosynthetic rates of upstream Sulphur River periphyton and Selenastrum capricornutum Prinz. Pooled results of bioassay runs indicated a 20 per cent BKME concentration effectively reduced control ^{14}C-assimilation levels by 50 per cent.

The downstream reduction observed for in situ productivity was 5 per cent lower than that predicted by the color bioassay.

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CHAPTER I

INTRODUCTION

The southern United States is currently experiencing unprecedented population and economic growth. Much of this growth has been at the expense of the north-east and north-central regions of the country (U.S. Bureau of the Census, 1980). Whether the successful absorption of this migration is possible depends largely on the intelligent management of the region's freshwater resources (MacNeil, 1981).

A principal factor considered in allocating water resources for various societal needs is water quality (Teclaff and Teclaff, 1973). In most cases, the better the water quality of an aquatic system, the wider its scope for potential use; i.e., the concept of a multi-use resource. Therefore, efficient and effective management of water quality would necessitate the maintenance and possibly improvement of aquatic system integrity for the benefit of society as a whole.

Water quality management can best be accomplished with knowledge of an aquatic ecosystem's current quality and assimilative limitations (Cairns, 1976). Complexities of aquatic ecosystems do not always allow these limitations to be easily identified (Hynes, 1970). However, an assessment of an ecosystem's ability to resist alteration can be accomplished by identifying and quantifying its structural and functional

characteristics and monitoring responses of these characteristics to a particular perturbation (Barret et al. 1976; Cairns, 1976).

The Pulp and Paper Industry

Water Resource Requirements

One of the largest demands on water resource allocation and integrity in the southern United States is the pulp and paper industry (Figure 1). Sixty-four per cent of the nation's pulp is produced in this region, primarily via the kraft process (Department of Commerce, 1981). The average water-use rate for a typical southern kraft mill is 190 m^3 for every metric ton of bleached pulp and paper produced. For a large plant, this could be as high as 240,000 m^3 per day. Even though present pulp and paper process technology recycles much of the water used (Saltman, 1978), waste effluent volumes are typically 140,000 m^3 per day (Rainville et al. 1975).

The aquatic systems which are used to provide processing waters usually receive the mills' wastewater discharge. Many pulp mills in North America are situated near estuaries. Although these mills do not compete with other freshwater uses, they typically do not employ wastewater treatment and can have a serious impact on the estuarine environments to which they discharge (Hodges, 1973; Parker and Sibert, 1973, 1976). Inland pulp mills, typical of the South, necessarily compete with other freshwater uses, e.g., municipal supply. Additionally,

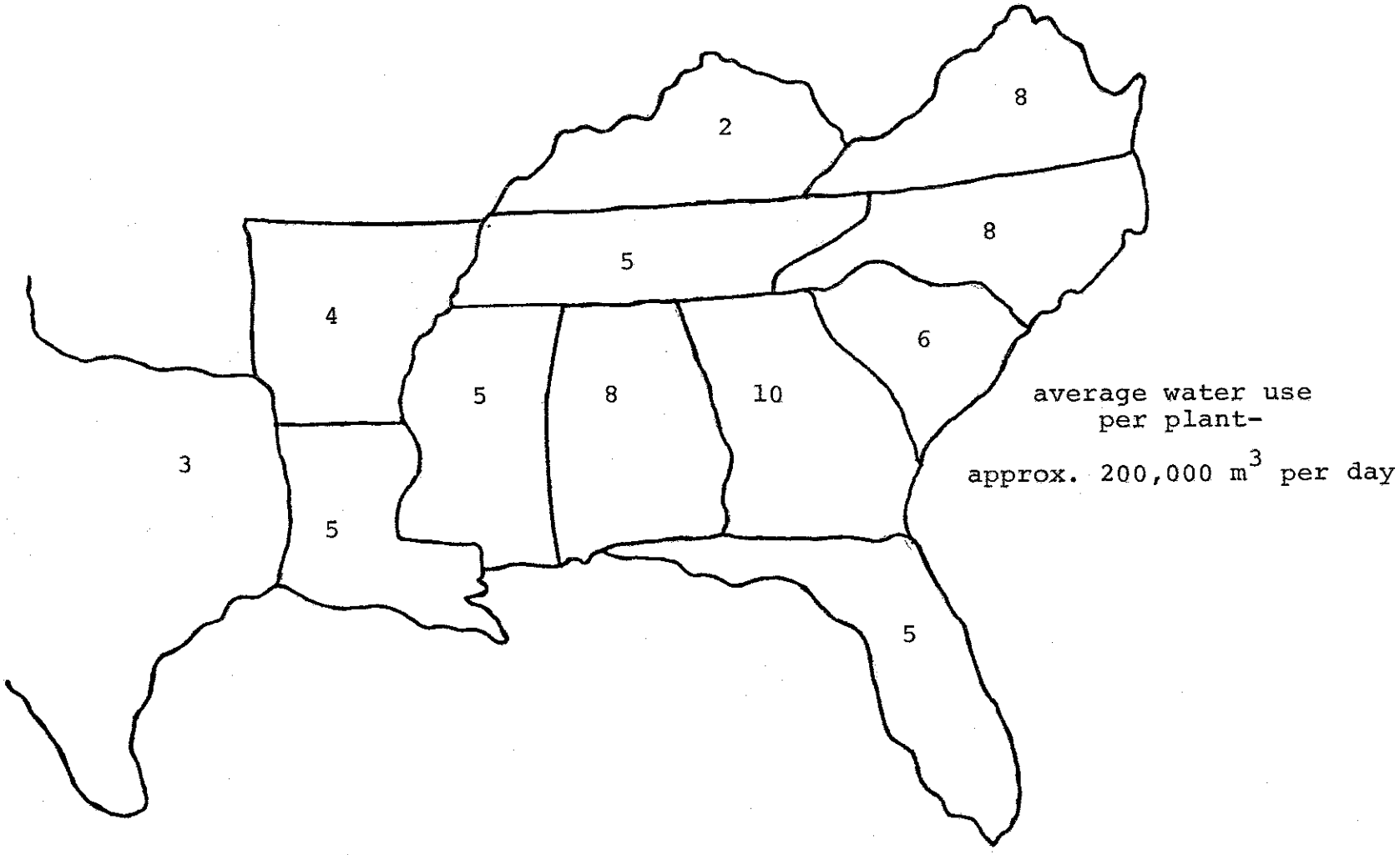


Figure 1. The number of pulp and integrated pulp and paper mills in the southern United States. (Data from the U.S. Department of Commerce, 1977)

the potential exists to impair the water quality for other uses downstream.

Kraft Processing and Waste Characteristics

The impacts of pulp and paper mill effluents on the quality of aquatic environments are complex and result from the interaction of several potentially adverse waste characteristics. These include toxicity, biochemical oxygen demand (BOD), pH, suspended and dissolved solids, and color (Walden, 1976). The relative contribution of each aspect to the overall impact varies considerably with the pulping process and its efficiency, the species of wood pulped, the waste treatment employed, and the physical-chemical characteristics of the receiving streams (Hutchins, 1979).

The following description of the kraft processing method is a simplified presentation of a highly complex and technologically intense manufacturing process (Davis, 1975; Saltman, 1978; Rainville et al. 1975; Hutchins, 1979). In southern mills, conifers are the principal source of pulp. Kraft pulp is produced by digestion of wood chips in sodium sulfide and sodium hydroxide under heat and pressure (Figure 2). As a result, lignins and other wood extractives are separated from the cellulose fibers, and stain the pulping solution a black color. A high percentage of the pulping chemicals can then be recovered from this "black liquor" by evaporation and burning. Washings of the impure pulp comprise most of the

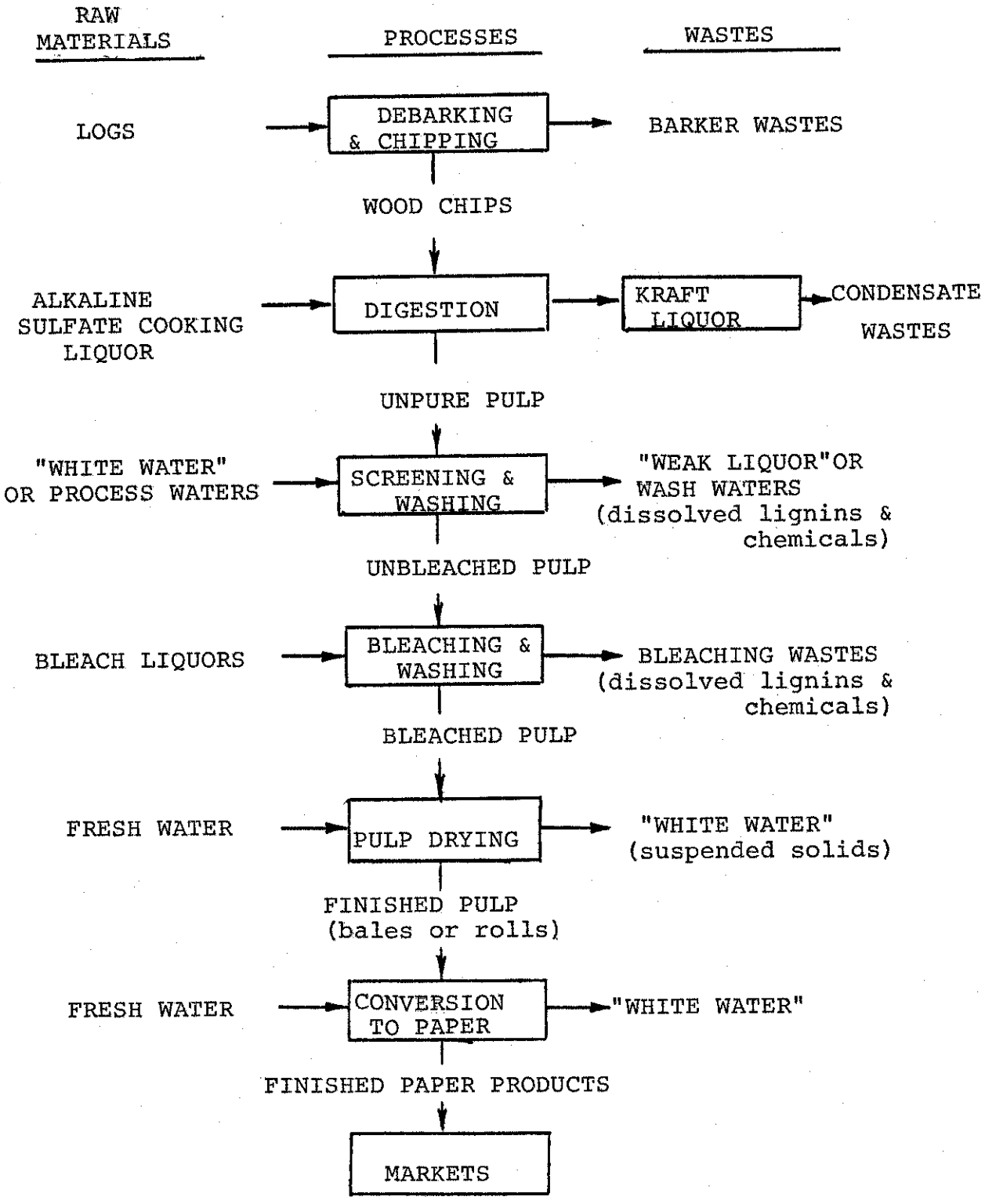


Figure 2. Schematic of the bleached-kraft pulp process (from Hutchins, 1979)

effluent volume. As shown in Figure 2, other contributing waste streams originate from the recovery and bleaching processes. A series of bleachings, extractions, washings, and dryings of the impure pulp is then necessary to produce white paper. Sodium hypochlorite and chlorine dioxide are the common bleaching agents used, while caustic sodium hydroxide is used in extracting solutions.

Present pulp and paper mill waste treatment includes primary and secondary methods (Saltman, 1978). Primary stages consist of settling basins and clarifiers that serve to decrease suspended solids concentrations. At this point, pH is adjusted to neutral with lime which also precipitates sulfates as calcium sulfate. Secondary waste treatment is typically comprised of aerated-stabilization ponds. This biological treatment method has shown the capacity to greatly reduce toxicity and BOD concentration (Seim et al. 1977; Rainville et al. 1975).

The most extensively researched aspect of pulp and paper mill waste has been its toxicity to aquatic organisms, particularly fish (Hutchins, 1979; Walden, 1976). These studies employed toxicity bioassays on species from all trophic levels to determine their respective tolerances to various effluent types. Likewise, identification of toxic chemical constituents and their levels of lethality have been well studied (Leach and Thakore, 1975). Potential toxicants found in kraft mill wastes include chlorinated phenols, quinones, sulfides,

mercaptans, resins, and fatty acids. With the exception of fish species, sub-lethal effects on aquatic organisms are not well known (Hutchins, 1979). The results of these investigations provide valuable information concerning potential stress on the structure of aquatic biological communities. However, effects on the integrity of system functions can only be implied from these data (Mount, 1979).

Strategy of Impact Assessment

As previously noted, assessments of water quality or environmental impacts are best accomplished when both structural and functional aspects can be identified and monitored (Barret, et al. 1976; Cairns, 1976). Aquatic ecosystem structure and function are concepts well-based in the development of ecology as a scientific discipline and evolved from efforts to describe and measure energy flow through levels of biological organization, i.e., trophic structure (Lindemann, 1942; Hutchinson, 1967; Odum, 1956; Margalef, 1963). Rodgers, et al. (1979) defined structure as,

> . . . any characteristic of the abiotic or biotic components of the system at any point in time that is related to the quantity, composition or quality, arrangement, and distribution or pattern of organization

and function as, ". . . any rate process of the system or its components."

Some examples of structure include

1) abiotic -- suspended solids concentration, temperature, and light attenuation;
2) biotic -- biomass, species lists, and diversity indices.

Examples of aquatic ecosystem function are:

1) abiotic -- sedimentation, reaeration coefficients, and flushing time;
2) biotic -- primary productivity, respiration, and species colonization rate.

Methods for measuring biological structure and function of aquatic environments provide information at two organizational levels:

1) organism or species -- level analyses, e.g., diversity indices and species colonization rate;
2) community or systems -- level analyses, e.g., chlorophyll _a_ and primary productivity.

In actuality, a complete characterization of aquatic ecosystem structure and function is improbable, if not impossible. However, Odum (1977) has suggested that a primarily systems-level approach can provide adequate information for intelligent impact assessment. This approach is twofold:

1) The measurement of functional, systems-level variables should predominate. The justification is that systems-level functions reflect the

integrated results of biotic and abiotic components interactions and interrelationships, thus providing the most insight to system integrity for the least effort. Odum (1977) stressed the measurement of photosynthesis and respiration as the most informative of systems-level functions.

2) Concominant measurement of ecosystem structural components should be made for specially selected, site-specific interests. These analyses may be systems- or species-level properties; e.g., levels of chlorinated hydrocarbons or the diversity of aquatic vegetation.

An example of the above approach is an impact assessment of urban and commercial development on Lake Tahoe quality by Tilzer, et al. (1976). In their study, system functions of phytoplankton productivity and sediment inflow were monitored with changes in system structural components - light attenuation and nutrient concentrations.

The systems-level strategy for impact assessment was used in the following study of a southern river system receiving waste effluent from a bleach-kraft pulp and paper mill.

The International Paper Company Texarkana Mill

International Paper Company's (IP) Texarkana bleach-kraft pulp and paper mill is located on the southern bank

of the lower Sulphur River, 0.2 km upstream of the Arkansas state line, in Cass County, Texas (latitude $33^{o}18'$, longitude $94^{o}5'$) (Figure 3). The mill has been in operation since 1972 and pulps sixty per cent slash and short-leaf pine and forty per cent mixed hardwoods to produce notebook, butcher, and bathroom paper products (Phil White, personal communication). Processing water for the mill is taken from Wright Patman Dam, 33 km upstream of the plant. Combined-stream waste effluents are 1.6 $m^3 \cdot sec^{-1}$ from an average production of 1270 metric tons of bleached pulp daily. The bleach-kraft mill effluent (BKME) undergoes secondary treatment in approximately 690 hectares of aeration-stabilization lagoons. This treatment facility has a holding capacity of 3.80×10^9 m^3 and is capable of removing eighty per cent of the BOD (Phil White, personal communication). The mill is permitted to discharge its waste by the Texas Department of Water Resources. The BKME water quality regulated by the agency include BOD, total suspended solids, chlorides, sulphates, and pH (TDWR permit #01339). In addition, minimum dissolved oxygen (DO) levels are prescribed for the lower Sulphur River. Effluent color is currently not regulated, and its possible effects on the quality of aquatic environments are still relatively unknown (Hutchins, 1979). Secondary treatment does not signficantly remove effluent color since organic compounds responsible for coloring BKME, such as lignin sulfonates

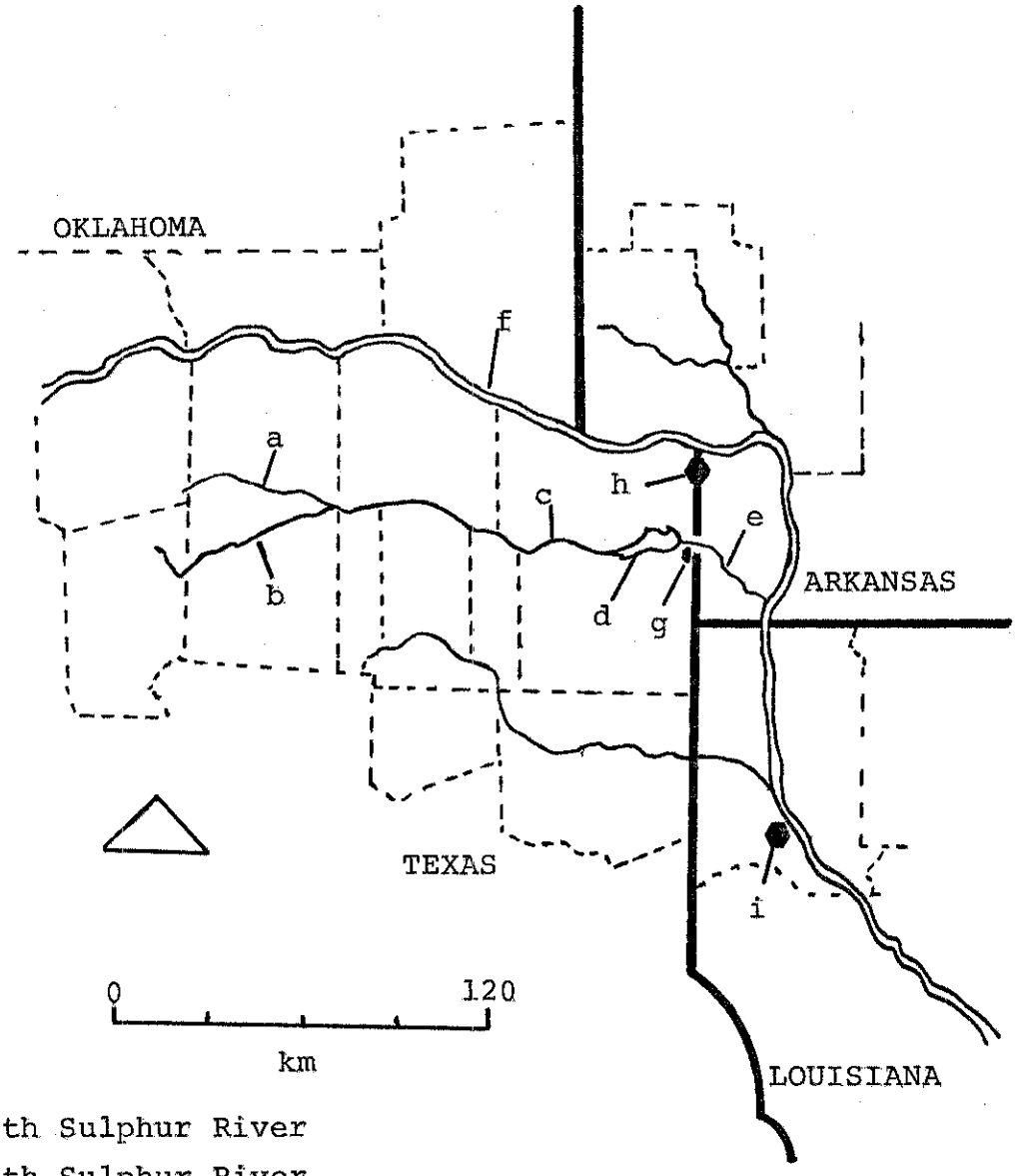
and other wood extractives, are highly resistant to bacterial degradation (Dugan, 1974; Wong and Prahacs, 1977). Tertiary, physical-chemical treatment methods, e.g., activated carbon and ozone, have proven effective, but are cost-prohibitive to large scale mill operations (Wong and Prahacs, 1977).

Sulphur River Basin

The Sulphur River system is part of the Red River Basin (Figure 3) and has a drainage area of approximately 1.6 x 10^4 km^2 (Texas Interagency Natural Resources Council, 1970). It consists of the upper Sulphur River, Lake Wright Patman, and the lower Sulphur River.

The upper Sulphur River is composed of the North and South forks, respectively originating in Fannin and Hunt Counties, Texas. The North and South forks join in Hopkins County, and the upper Sulphur River then flows east to Wright Patman Dam, forming Lake Wright Patman, 14.4 km southwest of Texarkana, Texas. Impoundment of the river began in 1953 for flood control and as a municipal water supply for Texarkana. Lake Wright Patman, maximum capacity of 7.10 x 10^9 m^3, was built and is operated by the U.S. Army Corps of Engineers.

The lower Sulphur River flows southeast from Wright Patman Dam for approximately 74 km until it joins the Red River in Arkansas. This reach of the Sulphur River system is a



a. North Sulphur River
b. South Sulphur River
c. Upper Sulphur River
d. Lake Wright Patman
e. Lower Sulphur River
f. Red River
g. International Paper Company, Texarkana Mill
h. Texarkana
i. Shreveport

Figure 3. Sulphur River basin

regulated stream whose flow fluctuates greatly on a yearly basis. Low flows are typically 0.28 to 8.5 $m^3 \cdot sec^{-1}$, occurring mainly during summer months. From October to April, the Corps releases larger volumes of up to and exceeding 283 $m^3 \cdot sec^{-1}$. This large variation in regulated flows characterizes the hydrology of the lower Sulphur River as very dynamic and somewhat unusual (Leopold et al. 1964; Ward and Stanford, 1979).

The Texarkana mill is the only industry on this segment. However, Days Creek, joining the Sulphur River 14.4 km below the mill's outfall, carries municipal waste from the city of Texarkana (Texas Department of Water Resources, 1981).

Primary Productivity

The Sulphur River's response to BKME was monitored by measurement of primary productivity. Primary productivity can be defined as the rate that radiant energy is stored as chemical energy, in the form of organic substances, by photosynthetic or chemosynthetic producer organisms (Odum, 1971). Net productivity is the total rate of organic matter production (gross productivity) minus producer respiration and represents the foundation of community trophic structure and dynamics, i.e., the quantity of organic matter available for consumers (Lindeman, 1942). In aquatic systems, the producer community is dominated by one or the other following

plant groups--phytoplankton, periphyton, and aquatic macrophytes (Wetzel, 1964, 1975).

Studies of the relationships of primary productivity to various environmental structural components--particularly light, temperature, nutrients, and photosynthetic standing crop--have produced volumes of published literature (Vollenweider, 1974; Goldman, 1969; Golterman, 1975; Wetzel, 1975). The knowledge of these interrelationships demonstrates the usefulness of primary productivity as an integrative tool for supplying system information (Odum, 1977). Its use is particularly well suited to BKME impact assessment for the following reasons:

1) BKME is known to have high concentrations of dissolved organics and suspended solids (Hutchins, 1977). These characteristics suggest that strong, and perhaps selective, light attenuation can be expected by absorption and scattering (Talling, 1957; Golterman, 1975; Spence, et al. 1971; Wetzel, 1975). Alterations in the Sulphur River light regime by BKME should be indicated by changes in primary productivity since photosynthetic rates are highly dependent on light availability (Vollenweider, 1974).
2) Positive or negative responses of primary productivity rates and standing crop are possible from

potential BKME nutrient enhancement or toxic effects, respectively (Rainville, et al. 1975; Bothwell and Stockner, 1980).

Pulp and Paper Mill Impacts on Primary Productivity

Primary productivity studies have been shown to provide valuable information for assessing impacts of pollution on aquatic environments (Rodgers, et al. 1979; Edmundson, 1970). However, very few studies have monitored the impact of mill wastes on natural populations of primary producers (Stockner and Cliff, 1976; Moore and Love, 1977).

The few studies of kraft pulp mill effects on primary production have attributed their respective results to one of three major impacts: 1) light attenuation from color; 2) phytotoxicity; 3) or eutrophication (nutrient enhancement). It is also interesting to note that all but one of these studies considered the effects of untreated, unbleached, kraft mill effluent (KME) on primary producers. Parker and Sibert (1976) and Stockner and Cliff (1976) investigated in situ phytoplankton responses to KME in the coastal waters of British Columbia. Stockner and Costella (1976) used axenic cultures of marine phytoplankton in laboratory toxicity studies of KME from British Columbia mills and found high molecular weight lignin derivatives to be inhibitory to growth. However, it was the consensus

conclusion of these marine studies that light attenuation was the overriding factor for observed decreases in natural phytoplankton photosynthesis. The results of a study by Mechenich (unpublished thesis, 1980) on the effect of color on phytoplankton in Lake DuBay, Wisconsin, concur with those above. She found that photosynthetic rates increased when lake water color was reduced, allowing higher light penetration. Different conclusions were presented by Moore and Love (1977), who tested KME effects on phytoplankton and periphyton populations in Nipigon Bay, Lake Superior. They determined that low concentrations of KME and low pH depressed photosynthesis as a result of toxic effects rather than light attenuation. Bothwell and Stockner (1980) assessed the influence of secondarily-treated BKME on periphyton from the McKenzie River, Oregon. They used on-site artificial streams and observed a nutrient enhancement effect; i.e., increased growth with increasing wastewater concentration. Apparently, light attenuation was not a factor in this study as a result of very shallow flows through their streams. Rainville, et al. (1975) used Coccochloris elebans, an estuarine phytoplankter, in laboratory bioassays to determine the toxicity of KME and BKME. Coccochloris' growth in the waste effluents, before and after various waste treatments, was plotted. From the results, they determined that toxicity of KME and BKME is

insignificant when appropriate waste treatement is used. The KME and BKME tested in Rainville's study were collected from several pulp and paper mills in the southern United States. However, there are no known published assessments of secondarily treated BKME impacts on in situ freshwater primary productivity in this or any other geographic region.

In 1979, the Institute of Paper Chemistry assessed the impact of the IP Texarkana mill effluent on Sulphur River periphyton community structure (unpublished report, 1980). The results of two samplings indicated no significant alteration in periphyton community structure at downstream sites relative to upstream reference sites. However, during the first sampling, substrates downstream of the mill outfall were not exposed to BKME for five days prior to recovery. Five days is sufficient time for periphyton to respond to a changed physical-chemical regime; therefore, it is unlikely that these samples adequately represent communities influenced by mill discharge (Patrick, 1971). Periphyton samples from the second sampling had been exposed to continuous discharge; however, the substrates were poorly colonized and were not analyzed. Primary productivity was not measured in this study.

The literature indicates that impacts to primary productivity by the pulp and paper industry are somewhat site-specific. Applying this information to the IP

Texarkana operation, one might expect

1) the secondary waste treatement system lessens any potential toxic impact; and
2) the light-attenuation from BKME color may have a significant impact.

Results from Bothwell and Stockner's study (1980) suggest nutrient enhancement may also have an effect on primary production in the Sulphur River. However, their experiments monitored growth, not photosynthesis. Additionally, they noted changes in species composition with increasing waste concentrations and postulated compensatory species selection. These observations leave unanswered the question of whether photosynthetic levels are maintained below IP Texakrana's discharge.

Objectives and Hypotheses

The objectives of this study were to assess the impact of BKME on structure and function of in situ primary production in the lower Sulphur River and to determine whether laboratory light-attenuation bioassays were useful in estimating BKME impact on in situ periphyton photosynthetic rates.

To accomplish these objectives, systems-level parameters of primary productivity were measured in field and laboratory experiments. Upstream-reference versus downstream-experimental sites were used in field studies and modeled in

laboratory bioassays. The purpose of the laboratory bioassay studies was to develop an integrative, predictive dose-response model of primary productivity. As suggested by Barret et al. (1976), such models should be a major end result of perturbation studies.

The following hypotheses indicate the three possible results of each parameter's upstream versus downstream comparison; i.e., increased, decreased, or unchanged downstream relative to upstream (Odum, et al. 1979). In addition, hypotheses of possible correlations of productivity parameters with selected environmental variables are stated.

Field Studies

1. H_o: Primary productivity of periphyton ($mgC \cdot m^{-2} \cdot hr^{-1}$) is not altered below the IP discharge relative to upstream stations.

   H_a: Periphyton productivity is subsidized downstream relative to upstream references.

   H_b: Periphyton productivity is decreased downstream relative to upstream references.

The use of periphyton in monitoring and assessing chemical and physical impacts on waste quality is extensive and well-documented (Patrick, 1973; Collins and Weber, 1975). Species lists, diversity indices, and other taxonomic, structural descriptions of periphyton communities have been

used to determine the extent and directionality of perturbations. Application of *in situ* measurements of periphyton productivity rates to assess perturbations in lotic systems are rare, presumably due to the difficulty in measuring metabolic rates in flowing systems (Rodgers, *et al*. 1979; Hynes, 1970; Benfield, 1981). However, recent advances in methodology have adapted ^{14}C-assimilation procedure to measurement of periphyton productivity with much success (Rodgers, *et al*. 1978; Rodgers and Harvey, 1976).

The measurement of periphyton ^{14}C-photosynthetic rates is particularly well-suited to the study of possible BKME impact on Sulphur River primary productivity for the following reasons:

1) The use of periphyton chlorophyll *a* as a measure of productivity may not be valid since its concentration is known to vary with light intensities as well as nutrient regimes (Wetzel, 1975).
2) The results of a taxonomic study do not necessarily reflect a change in functional levels.
3) ^{14}C-productivity methods have been shown to be 50 to 100 times more sensitive than dissolved oxygen methods (Wetzel, 1975).
4) Any sensitivity in the O_2 method would be seriously reduced in the presence of BKME oxygen demand.

5) Lack of consistent, natural sampling regimes in hydrologically unusual systems like the lower Sulphur River support the use of artificial substrates for replicate samples of periphytic communities.

2. H_o: The ratio of periphyton productivity to unit chlorophyll *a* ($mgC \cdot hr^{-1}/mg$ chl *a*) maintains its proportionality below IP discharge relative to upstream stations.

   H_a: The above ratio increases downstream relative to upstream references as a result of increased productivity rates and/or decreased chlorophyll *a*.

   H_b: The above ratio decreases downstream relative to upstream references as a result of decreased productivity rates and/or increased chlorophyll *a*.

The purpose in calculating this productivity:biomass ratio is to obtain an indication of relative productivity efficiency (McIntire and Phinney, 1965; Rosemarin, 1975; Platt and Filion, 1973; Brylinsky and Mann, 1973). Justification for calculating productivity efficiency (PE) lies in the assumptions that periphyton chlorophyll *a*, at the time of sampling:

1) represents an integrated response to factors controlling algal growth during substrate incubation; and
2) estimates the biomass of the photoautotrophic segment of the periphyton community.

Since a unit of time is included in the ratio (hr^{-1}), PE can represent a relative estimate of carbon turnover rates between upstream and downstream sites. Also, insights into community dynamics can be gained since variation in the ratio can be identified as differences in function (productivity) or differences in structure (algal biomass).

3. H_o: The structural index (mg chl a/mg ash-free dry weight) of the periphyton community maintains its proportionality below the IP discharge relative to the upstream reference stations.

   H_a: The structural index increases downstream relative to upstream references as a result of increased chlorophyll a and/or decreased ash-free dry weight.

   H_b: The structural index decreases downstream realtive to upstream stations as a result of decreased chlorophyll a and/or increased ash-free dry weight.

The structural index is very similar to the Trophic Index proposed by Clark *et al.* (1979) as an additional means of water quality assessment. The only difference between the two ratios is that Clark's Trophic Index is unitless since both chlorophyll *a* and ash-free dry weight are expressed in $g \cdot m^{-2}$ organic carbon.

Increases or decreases in the index represent compositional shifts in the community toward dominance by autotrophs or heterotrophs, respectively. These shifts can provide valuable systems information if correlated with some abiotic factor. For instance, a decreased index might indicate an influx of allocthanous organic material, shifting the index towards heterotrophic metabolism. High flows or current speeds might scour the substrate of detrital buildup, selecting for organisms with anchoring structures or strategies common to periphytic algae, thereby increasing the index value.

4. H_o: Phytoplankton primary productivity ($mgC \cdot m^{-3} \cdot hr^{-1}$) is not altered below the IP outfall relative to upstream reference stations.

   H_a: Phytoplankton productivity is subsidized downstream relative to upstream references.

   H_b: Phytoplankton productivity is decreased downstream relative to upstream reference stations.

The contribution of phytoplankton to lotic primary productivity has been the subject of controversy (Cummins, 1974; Minshall, 1978). Proponents of the river continuum theory suggest that free-floating plankton have little influence on carbon cycling in a flowing system (Vannote, et al. 1980). Phytoplankton biomass production is considered lost to stream processing as export except in high order segments where current speeds slow and turbidity shades benthic producers. However, a regulated stream as temporally and spatially dynamic in its hydrology as the Sulphur River defies general classification in the characteristic terms of the river continuum concept. Therefore, the unpredictable nature of this system seems to warrant an assessment of in situ phytoplankton productivity.

5. H_o: The ratio of phytoplankton productivity to unit phytoplankton chlorophyll a ($mgC \cdot hr^{-1}/mg$ chla) maintains its proportionality below the IP outfall relative to upstream stations.

   H_a: The above PE ratio increases downstream relative to upstream reference sites as a result of increased productivity rate and/or decreased chlorophyll a concentrations.

   H_b: The above phytoplankton PE decreases downstream

compared to upstream reference sites as a result of decreased productivity and/or increased chlorophyll a concentrations.

The purposes and justifications for calculating a phytoplankton PE ratio are the same as those discussed for the periphyton PE.

Laboratory Studies

6. H_o: There is no significant difference between periphyton ^{14}C-productivity rates ($mgC \cdot m^{-2} \cdot hr^{-1}$) measured in control and wastewater dilution groups.

   H_a: Periphyton ^{14}C-productivity rates increase relative to the control with increasing wastewater concentration.

   H_b: Periphyton ^{14}C-productivity rates decrease relative to the control with increasing wastewater concentration.

These hypotheses refer to a bioassay modeling the potential light-attenuating effect of BKME on stream photosynthesis (Dickson and Rodgers, 1980). In addition to Sulphur River periphyton, the responses of Selenastrum capricornutum Printz. were monitored in the bioassay. Therefore, the above hypotheses are applicable to Selenastrum ^{14}C-productivity as well. In this particular

bioassay, the above test organisms were not in direct contact with BKME, but were exposed to the relative light-absorption differences of diluted and undiluted wastewater concentrations.

The potential stress of chemical toxicity is removed in this design; therefore, only the effect of a BKME-influenced light regime on photosynthetic rates was measured (see CHAPTER II).

7. H_o: Phytoplankton, sampled from respective Sulphur River study sites and incubated under standard conditions of temperature and light, show no difference in measured ^{14}C-productivity rates ($mgC \cdot m^{-3} \cdot hr^{-1}$) between upstream and downstream samples.

   H_a: Standard incubation, phytoplankton rates of downstream samples are significantly higher relative to reference samples.

   H_b: Standard incubation, phytoplankton rates of downstream samples are significantly lower relative to reference samples.

This procedure was performed as a check for normal variations between stations due to possible differences of temperature and shading. These experiments are described in detail in the next chapter.

8. H_o: Physical-chemical environmental variables are not correlated with primary productivity parameters.

   H_a: Productivity parameters decrease with increasing light extinction.

   H_b: Productivity parameters decrease with increasing concentrations of organics and suspended particulates.

   H_c: Productivity parameters decrease with increasing color values.

   H_d: Periphyton structural parameters decrease with decreasing current speed.

The above relationships are those expected based on the literature review of BKME impacts on primary productivity. However, it is acknowledged that the converse of each alternate hypothesis is possible.

Environmental factors of principal interest to this study were those that indicate or influence the quantity and quality of photosynthetically available radiation (PAR). These included direct measurements of light energy attenuation through the water column; commonly expressed as an extinction coefficient, ε (Talling, 1957; Spence et al. 1971; Golterman, 1975). Also, water chemistry parameters known to absorb or scatter light were selected for correlation analysis. These were measurements of

dissolved or suspended materials; e.g., total dissolved and suspended solids, total and dissolved organic carbon, turbidity, BOD, and true and apparent color (Wetzel, 1975; Tilzer et al. 1976; DiToro, 1978). These parameters were considered indicative of BKME presence if their values were found to be higher downstream during discharge (Hutchins, 1979).

The relationship of periphyton structure to current velocity was also tested. Current speed is known to affect both the type and quantity of substrate colonization (Whitton, 1975; Weitzel, 1979). The major assumption made in performing this analysis is that velocities maintained relatively constant levels at each site during the incubation period.

Nutrients were not tested for significant correlations in this study since appropriate uptake measurements were not performed (Bothwell and Stockner, 1980).

9. H_o: Changes in primary productivity rates measured in the light-attenuation bioassay do not predict in situ rates changes observed downstream during mill discharge.

   H_a: The changes in in situ primary productivity rates observed at downstream sites are similar in magnitude and direction to those predicted by the light attenuation bioassay results.

The percent dilution of mill discharge by the regulated flow from Wright Patman Dam was the basis for comparing the bioassay results with those measured in the Sulphur River. Probit analysis of the bioassay data produces a probability curve from which the responses of the test organisms can be predicted for any particular dose within the range of doses tested (Finney, 1952; Sprague, 1973). In this study, the dose was the various dilutions of BKME with upstream Sulphur River water, and the responses of the test algae--Sulphur River periphyton and Selenastrum capricornutum Prinz.--were their respective photosynthetic rates.

With probit analysis, a prediction can be made of in situ rates downstream of mill discharge from the calculated concentration of BKME to which the indigenous primary producers were exposed. To test the above hypotheses, statistical comparison of the predicted and observed rates can be accomplished with Chi-Square analysis.

If bioassay rates decrease with increasing BKME concentration, probit analysis can be used to calculate an ED_{50}; i.e., the dilution that effects a fifty per cent reduction in photosynthetic rate. The purpose of calculating ED_{50} in similar to that of LC_{50} determinations for toxicity bioassays. This value serves as a descriptor of the bioassay results and allows comparisons with other tests for monitoring or hazard assessments (Sprague, 1973; Maki, 1979; Kimerle et al. 1978).

CHAPTER II

MATERIALS AND METHODS

Primary productivity studies were conducted on the lower Sulphur in July and October, 1980 and January, April, July, and October, 1981. These studies included in situ and laboratory experiments.

Field Experiments

Productivity Stations

In July of 1980, five stations were chosen as sites for primary productivity studies (Figure 4). Two control stations were located upstream to represent river conditions unaffected by BKME. These were designated as Stations 2 and 2NT, 8.0 km and 0.8 km, respectively, upstream of the mill's outfall. Two stations were chosen 0.5 km downstream of the IP discharge. One each was located near the left bank and the right bank, and were designated as Stations 3L and 3R, respectively. The decision to assign left and right bank stations was based on the results of preliminary water quality surveys. The surveys had shown differences in physical-chemical parameters between the left and right banks and suggested that this section of the river represented the mill waste's mixing zone. Station 4 was placed

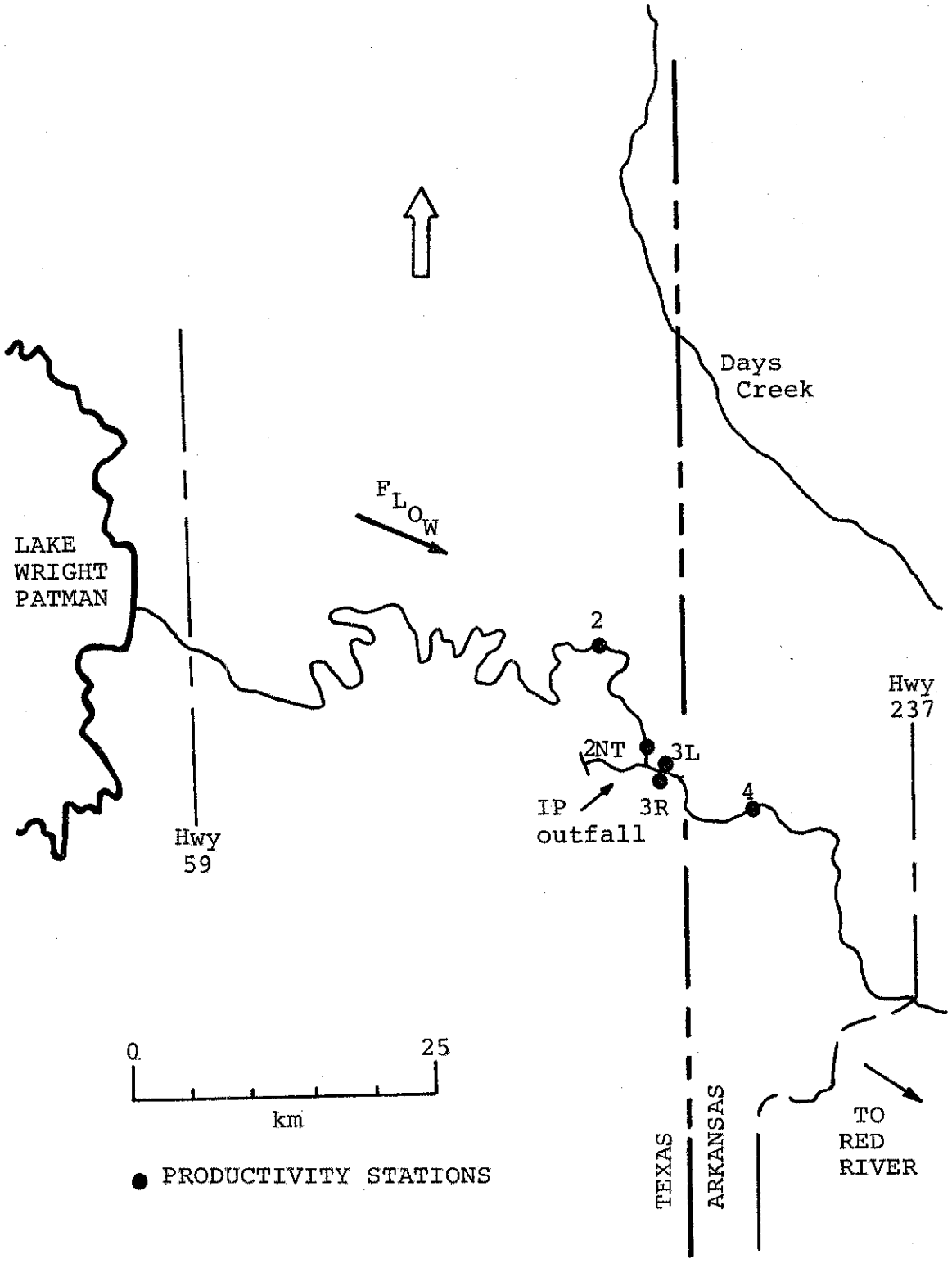


Figure 4. Study site

5.0 km downstream of the outfall and represented river conditions influenced by a thoroughly mixed BKME discharge.

Sampling Apparatus

One month prior to each sampling date, a periphytometer (artificial substrates for sampling indigenous periphyton) was set out at each of the five productivity stations. Periphytometers consisted of fifteen, 7.6 cm x 15.2 cm x 0.6 cm unglazed porcelain plates to provide the surface for algal colonization (Gerhardt, et al. 1977). The two versions of the periphytometer used for this study are shown in Figure 5A and 5B. The original periphytometer (Figure 5A) oriented the ceramic plates horizontally. However, this configuration accumulated an unmanageable amount of silt. This design was replaced after the July 1980 sampling by one with vertical plates (Figure 5B), thereby reducing the high silt load.

Each periphytometer held fifteen replicate plates: six replicates for in situ primary productivity measurements; three replicates for chlorophyll a extraction and determination; three replicates for ash-free dry weight estimates of biomass; and, three adenosine triphosphate (ATP) assay replicates. After the July 1980 survey, the ATP assay was dropped, and these three replicates were subsequently omitted.

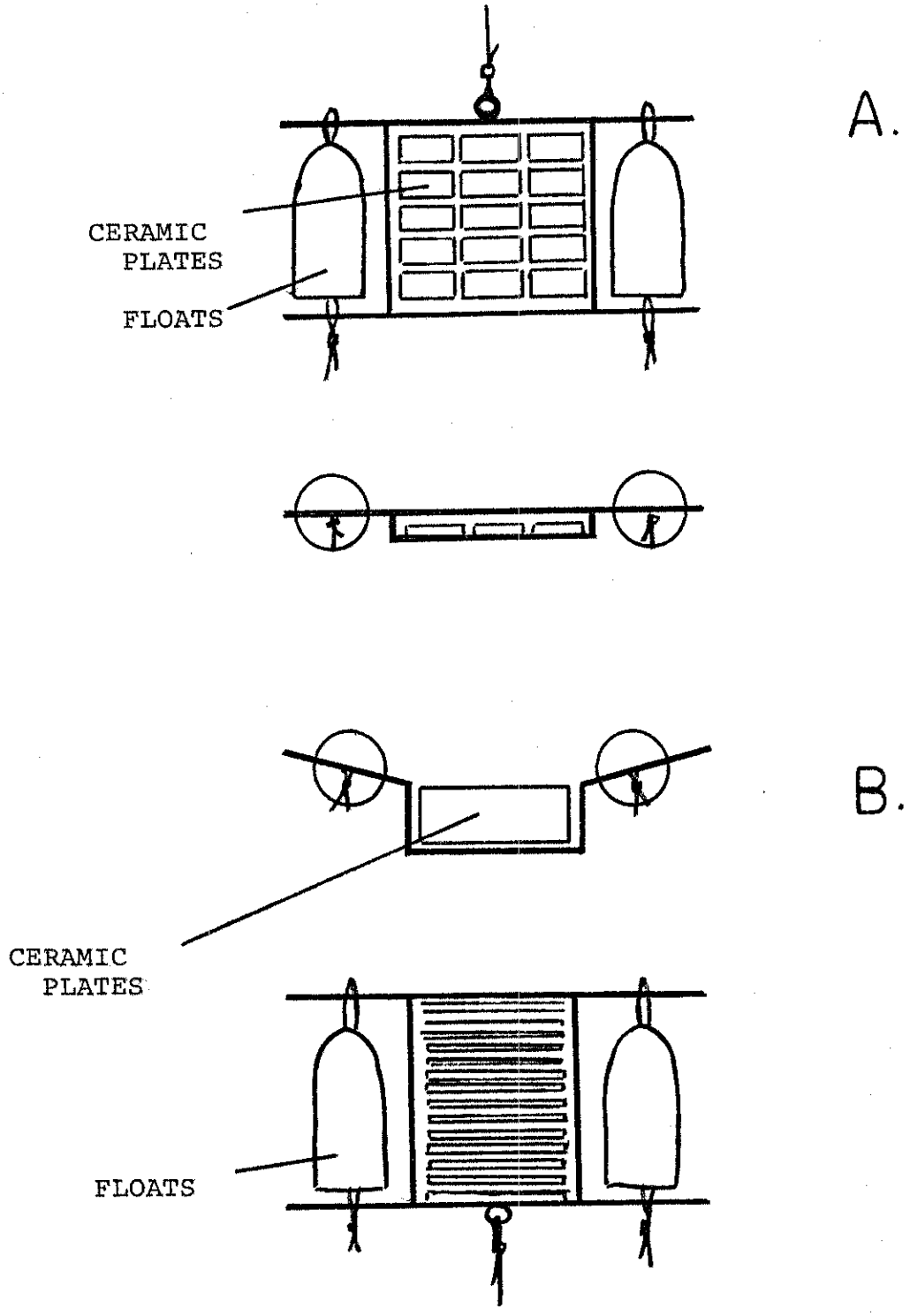


Figure 5. Periphytometers used for in situ measurements of periphyton productivity; A. original design; B. modified design (from Dickson and Rodgers, 1980).

The periphytometers were anchored at the respective stations as shown in Figure 6. The lead float aided in maintaining a level position in fast currents and in screening the periphytometer from debris.

In Situ ^{14}C Studies

Primary productivity at Sulphur River stations was measured using in situ incubation and ^{14}C methods. Since approximately 13.0 km separate Stations 2 and 4, two boat crews of two workers each were required to perform the experiments. One crew was assigned the upstream stations, and the other was responsible for stations downstream of the mill discharge. This arrangement allowed all stations to start their incubations within one-half hour of each other. At each station, both periphyton and phytoplankton samples were incubated simultaneously.

Six replicate ceramic plates with their complement of attached periphyton were carefully removed from the periphytometer, and each was placed into an incubation chamber filled with 1.9 ℓ of river water from the particular station. Three of the chambers were clear polystyrene and designated as light replicates, while the other three were opaque, dark chambers. Figure 7 shows the chambers which were essentially the same chamber designed by Rodgers, et al. (1978). The six chambers were then placed in an incubation rack that floated the chambers at a depth of 10.0 to 20.0 cm below the

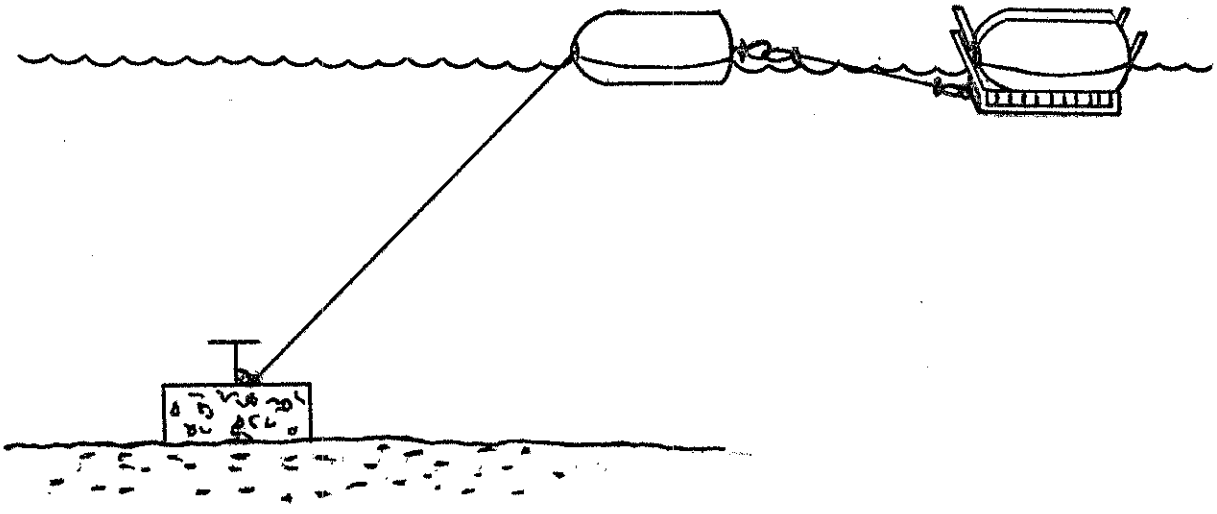


Figure 6. Periphytometer anchorage.

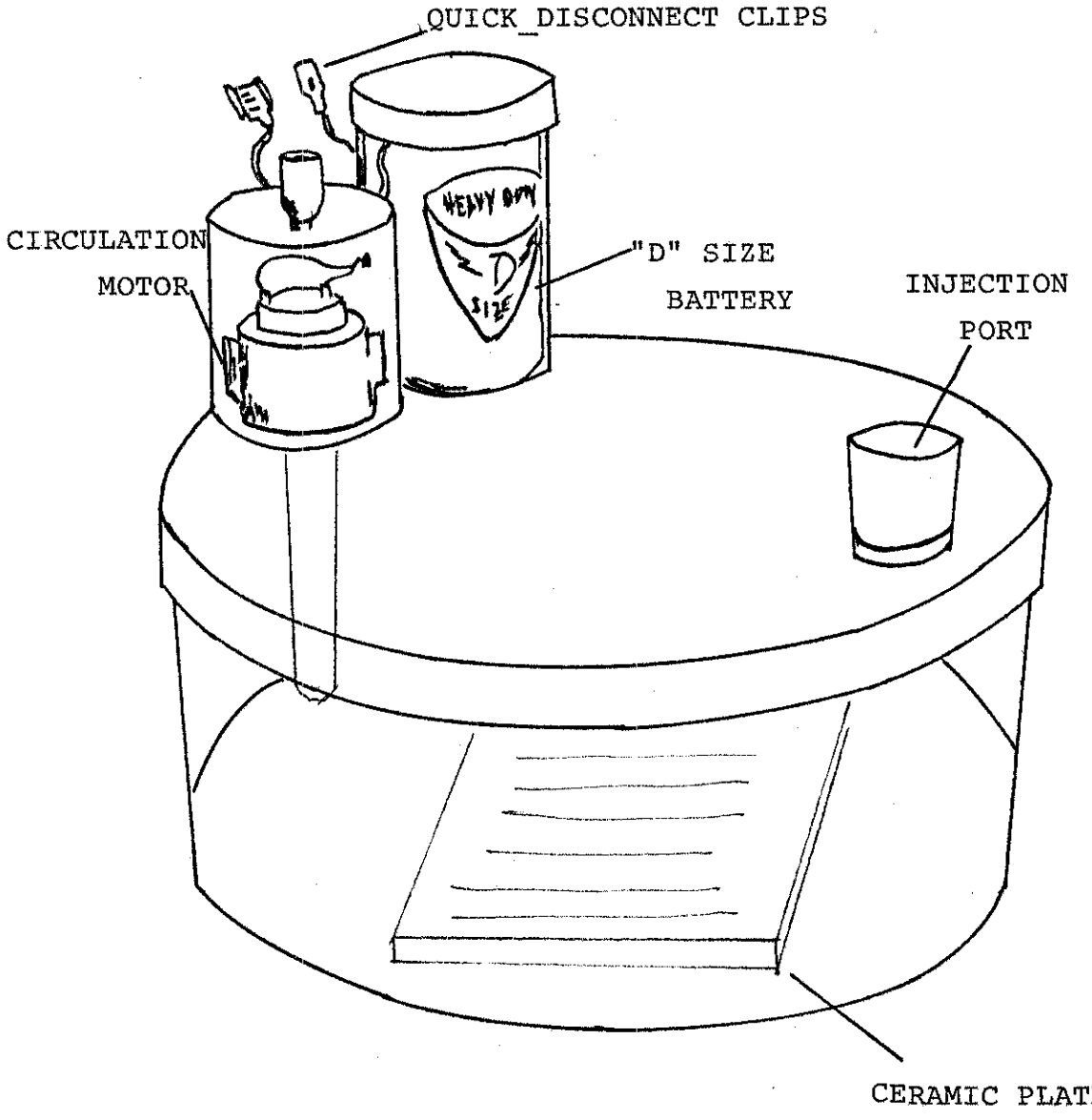


Figure 7. In situ periphyton ^{14}C-incubation chamber

surface. Circulation motors were started and two mℓ of $NaH^{14}CO_3$ (Amersham) solution (approximately ten μCuries $mℓ^{-1}$) were injected into each chamber. The incubation period began at the time of ^{14}C injection and continued for four hours.

The classical light and dark bottle ^{14}C method (Steeman-Nielson, 1952) of measuring planktonic productivity was performed at each station. Triplicate 300-mℓ Wheaton light and dark bottles were filled at the respective stations with river water and spiked with one mℓ of the ^{14}C solution. The bottles were incubated in yet another floating incubation rack for the same photoperiod as the periphyton samples. Incubation depth for the bottles was 5.0 cm.

One mℓ of 3N H_2SO_4 was injected into each phytoplankton bottle, and five mℓ of the acid were sprayed on the periphyton plates to terminate the ^{14}C experiments. In addition to halting the photosynthetic reaction, the lowered pH converts unassimilated inorganic carbon, both radioactive and normal isotope, to free CO_2. Bubbling the samples with air, as described later, drives out the gaseous CO_2, leaving only the radioactivity bound as organic compounds for assay (Schindler, 1972).

Light Measurements

To identify and correlate possible color effects of BKME on primary productivity, a major effort was made to measure the amount and character of light energy impinging on the experimental stations and penetrating their respective water columns. This was accomplished using several light-measuring instruments. A LI-COR LI-1776 Solar Monitor equipped with a LI-200SB Pyranometer Sensor recorded and stored total daily solar radiation data for the Texarkana mill area. Radiation recording began October 15, 1980 and continued throughout the study. The LI-200SB measures total energy in watts.m^{-2} ($W \cdot m^{-2}$) from a range of 400 to 1100 nm wavelengths. A portable Belfort 5-3850 pyranograph measured the total light energy contributed by a 280 to 2000 nm wavelength range in units of Langleys.min^{-1} ($ly \cdot min^{-1}$). This instrument was used to provide hourly light energy data on the river during *in situ* primary productivity experiments. A Protomatic submarine photometer measured incident and reflected light intensities at each station just below the surface and at 1.0 m depth. The photometer measures a 300- to 800-nm wavelength range of light intensity in foot-candles units (ft-c). Finally, the quality of the light energy penetrating the photic zone at each station was determined with a specially designed International Light (IL) 300 Research Radiometer. Ten individual light cells

each measure a 10 nm range of wavelengths within the ultraviolet-infrared spectrum. The ten ranges are 344-356 nm; 395-405 nm; 445-455 nm; 495-505 nm; 545-555 nm; 595-605 nm; 645-655 nm; 695-705 nm; 745-755 nm; and 795-805 nm. The IL 300 records light energy in units of watts$\cdot$cm^{-2} (W$\cdot$cm^{-2}). Each of these ranges was measured just beneath the surface and, when possible, at 1.0 m. All of the above light measurements were made during the primary productivity incubation period.

TABLE I

FACTORS USED TO CONVERT VARIOUS SOLAR RADIATION UNITS TO WATTS PER SQUARE METER

Instrument	Units	Conversion Factor	Reference
LI-200 SB Solar Monitor	W$\cdot$cm^{-2}	---	---
Belfort Pyranograph	ly$\cdot$cm^{-2}	= 698 W$\cdot$m^{-2}	(Wetzel, 1975)
IL-300 Spectro-radiometer	W$\cdot$cm^{-2}	= .0001 W$\cdot$m^{-2}	
Protomatic	ft-c		
sunlight		= .04 W$\cdot$m^{-2}	(Talling, 1957; Wetzel, 1975)
fluorescent (40 W, cool-white		= .0386	(Bickford and Dunn, 1972)

Table I shows the factors used to convert the recorded units for each light instrument to $W \cdot m^{-2}$. Protomatic and spectroradiometer surface and depth readings were then used to calculate vertical extinction coefficients for each station. Extinction, or attenuation, coefficients are calculated with the equation below and describe the rate at which light disappears through the water column (Talling, 1957; Vollenweider, 1974; Golterman, 1975).

$$\varepsilon = \frac{\ln I_o - \ln I_z}{z} \quad \text{(Wetzel, 1975)}$$

ε = extinction coefficient (m^{-1})

z = depth (m)

I_o = subsurface irradiance

I_z = irradiance at depth, z

Additional Sampling

Water samples containing phytoplankton were taken at the productivity stations and placed on ice. These samples were used to measure primary productivity under standard laboratory conditions.

Three replicate periphyton plates from each station for ash-free dry weight estimates were placed individually in plastic containers on ice and returned to the laboratory. The remaining three periphyton samples also were placed in plastic containers, and 10.0 mℓ of 90 per cent acetone (v/v)

added to the plate surfaces. These chlorophyll extractions were immediately iced.

One hundred and seventy liters of Station 2 river water and sixty liters of the mill's finished waste effluent were collected in polypropylene carboys and returned, at ambient temperature, to the laboratory. These water samples were used in making a wastewater dilution series for color bioassay experiments described later.

Laboratory Analyses

Water Chemistry

The importance of physical-chemical data to an investigation of this system's primary productivity cannot be overemphasized. Aside from the impact of BKME, temporal variation in Sulphur River primary productivity can be expected from seasonal and unusual hydrologic changes. These variations may be quantified and correlated with measured variations in the physical-chemical characteristics of the system (Vollenweider, 1974). Knowledge of these relationships is important for comparing variations in primary productivity to the influence of BKME. Therefore, water quality measurements were routinely performed for each of the productivity stations. In addition, chemical analysis was done on wastewater:river water dilutions for chemical parameters considered to best indicate the presence of mill effluent.

TABLE II

PHYSICAL AND CHEMICAL WATER QUALITY PARAMETERS
DETERMINED FOR EACH SURVEY AT EACH
SULPHUR RIVER STATION

Parameter	Method	Reference
DOC	Combustion-IR Detection	Standard Methods page 532
TOC	Combustion-IR Detection	Standard Methods page 532
BOD	Incubation, 5 days	Standard Methods page 543
Temperature	YSI meter	
Conductivity	YSI meter	
pH	YSI meter	
Chloride	Orion electrode	
Sulfate	Turbidimetric	Standard Methods page 496
NH_3-N	Orion electrode	
NO_3-N	Orion electrode	
Ortho PO_4-P	Ascorbic Acid	Standard Methods page 481
Total PO_4-P	Digestion	Standard Methods page 424
Turbidity	Turbidimeter	Standard Methods page 132
Hardness	Titration	Standard Methods page 202
Acidity	Titration	Standard Methods page 273
Alkalinty	Titraton	Standard Methods page 278
Dissolved Oxygen	YSI meter	
Color, Apparent	Visual comparison	Standard Methods page 64
Color, True	Visual comparison	Standard Methods page 64

Parameters underlined were determined in the field.

River water was collected at each station in triplicate, 1.0 ℓ surface grab samples and transported on ice to the North Texas State University Water Quality Laboratory for chemical analyses. Table II lists the physical-chemical parameters measured and the reference of the method used for each analysis.

Periphyton Biomass Estimates

Ash-free dry weight.--Each of three replicate periphyton samples collected at each station was scraped into a tared, 35.0 mℓ porcelain crucible and weighed on a Mettler H6 analytical balance for wet weight determination. The crucibles were previously combusted at 500°C for one hour in a Thermolyne muffle furnace, desiccated, and weighed. The samples were dried at 103°C in a Blue-M drying oven and desiccated to constant weight. The samples were then ashed at 500°C for one hour in the muffle furnace, desiccated, and ash weight recorded. Ash-free dry weight was calculated as follows: (Standard Methods, 14th edition)

$$g\ m^{-2} = \frac{(\text{dry weight} - \text{ash weight})}{\text{area of substrate } (m^2)}$$

Chlorophyll a.--The monochromatic method described in the 13th edition of Standard Methods was used to estimate chlorophyll a concentrations of replicate periphyton samples.

Ten mℓ of 90 per cent acetone (v/v) was added individually to three replicate plates for each station. Upon return to the laboratory, the samples were frozen for 18 to 24 hours. After thawing, the acetone extract was poured off into a 15.0 mℓ centrifuge tube and centrifuged at 3,000 rpm (800 x g) for five minutes in a Precision Vari-Hi-Speed clinical centrifuge. The clarified pigment extract was measured for absorbance at 665 nm wavelength in 1.0-cm pathlength quartz cuvettes in a Beckman Model 25 spectrophotometer. Chlorophyll a content was calculated as follows: (Standard Methods, 13th edition)

$$\text{mg chl } a \cdot m^{-2} = \frac{13.4\ D_{665} \times \text{volume of extract } (\ell)}{\text{area of substrate } (m^2)}$$

$$D_{665} = \text{absorbance at 665 nm}$$

Analysis of ^{14}C Phytoplankton Samples

Assimilation of ^{14}C by phytoplankton was determined by liquid scintillation counting. As mentioned, the plankton samples were acidified in the field. In the laboratory, a 5.0-mℓ subsample was transferred from each replicate bottle to a glass scintillation vial. The vial was then placed in a bubbling chamber (modified from Wessels and Birnbarn, 1979), and the subsamples vigorously bubbled for thirty minutes (Schlinder, 1972). Thirty-two subsamples could be bubbled at a time. Fifteen mℓs of Aquasol-II (New England

Nuclear) were added to each subsample, and the vials dark-adapted overnight. The dark adaption allowed time to quench chemical and/or light-stimulated scintillations before counting the samples. The samples were counted three times, for one minute each, in a Beckman LS-100 Liquid Scintillation System. Counting efficiencies were determined for all phytoplankton and periphyton samples. Three ^{14}C-toluene standards (New England Nuclear) for each type of scintillation sample were counted with the respective samples. The percentage of disintegrations per minute counted to the known quantity in the standards was used as the counting efficiency. Absolute phytoplankton productivity rates were calculated with the following equation: (modified from Standard Methods, 14th edition)

$$P_{\text{phytoplankton}} = \frac{{}^{14}C_f \times {}^{12}C_i \times 1.064}{{}^{14}C_i \times T}$$

$^{14}C_f$ = (cpm light-cpm dark) x 10^3 $m\ell \cdot \ell^{-1}$

$^{12}C_i$ = initial dissolved inorganic carbon ($mgC \cdot \ell^{-1}$)

$^{14}C_i$ = ^{14}C initially available (cpm)

T = incubation time (hr)

1.064 = isotopic correction factor for ^{14}C (Standard Methods, 14th edition)

Analysis of ^{14}C Periphyton Samples

Wet-oxidation procedure.--A modified wet-oxidation method was used to measure the amount of photosynthetically-fixed ^{14}C by attached algal communities (Shimshi, 1969). Briefly, a combination of concentrated chromic acid and 100°C temperature mineralized the organic matter of a periphyton sample to carbon dioxide and water vapor. Therefore, any radioactive carbon assimilated into organic matter during photosynthesis is then released as radioactive carbon dioxide ($^{14}CO_2$). Finally, the $^{14}CO_2$ is trapped in a 0.5N NaOH solution, Aquasol-II added, and the sample counted.

The specific steps of the wet-oxidation procedure are as follows:

1) the preserved periphyton samples were scraped off the ceramic plates with a single-edge razor blade into preweighed 50.0 mℓ beakers, and each sample's wet weight determined;
2) each beaker and its contents were placed into a 448 mℓ Mason jar;
3) a CO_2 trap, 3.5 mℓ of 0.5 N NaOH in a glass scintillation vial, was also placed in the jar;
4) concentrated chromic acid was added to the sample in a 10.0 mℓ per gram-wet-weight ratio, and the jar immediately sealed;

5) the sealed samples were incubated for one hour in an autoclave at 100°C without pressure (isothermal technique);
6) the jars were allowed to cool, opened, and the NaOH traps removed; and,
7) fifteen mℓs of Aquasol-II were then added to each vial-trap, and the contents of the vials counted as previously described.

The precision of the method was established by assaying subsamples of a replicate periphyton plate and found to be 92 per cent.

Harvey Oxidizer procedure.--Beginning with the April 1981 survey, periphyton productivity samples were oxidized for $^{14}CO_2$ recovery with a R. J. Harvey OX400 oxidizer. Unlike the wet-oxidation technique, the oxidizer mineralizes organic matter with exceedingly high temperatures (900°C), oxygen, and chemical catalysts in a combustion tube. Instead of an NaOH trapping solution, the radioactive and normal isotopic CO_2 released by combustion was trapped in OXIFLUOR-CO_2 (New England Nuclear), a trapping-scintillation mixture formulated for oxidizer use. A 0.5 to 1.0 g subsample from each replicate plate was transferred to a preweighed, fused-quartz glass boat. Subsampling was required as a result of sample volume limitations, and the precision of the subsampling was determined to be 97 per

cent. The boat and contents were then inserted into the combustion tube of the oxidizer. An oxygen-nitrogen gas mixture carried the resulting CO_2 and water vapor out of the oxidizer and into a condenser-trap containing 15.0 mℓ of OXIFLUOR-CO_2. The end of the condenser was then rinsed once into the scintillation vial with 3.0 mℓ of OXIFLUOR-CO_2. These traps were modified to accept a scintillation vial on the end (Figure 8). This increased time efficiency for running a multitude of samples and trapping efficiency by reducing loss of counts from rinsing the entire trap. Methanol was used to clean the traps and prevent carryover of radioactivity. Samples were counted as above on the Beckman LS-100 after dark adaption.

The trapping efficiency with the Harvey Oxidizer was twenty per cent higher than the wet-oxidation technique. Periphyton rates determined by wet-oxidation were, therefore, corrected upward for comparability. Absolute periphyton productivity rates were calculated with the following equation: (Rodgers, et al. 1978)

$$P\ periphyton = \frac{{}^{14}C_f \times {}^{12}C_i \times V \times 1.064}{{}^{14}C_i \times A \times T}$$

${}^{14}C_f$ = (cpm light-cpm dark)

${}^{12}C_i$ = initial dissolved inorganic cargon ($mgC \cdot \ell^{-1}$)

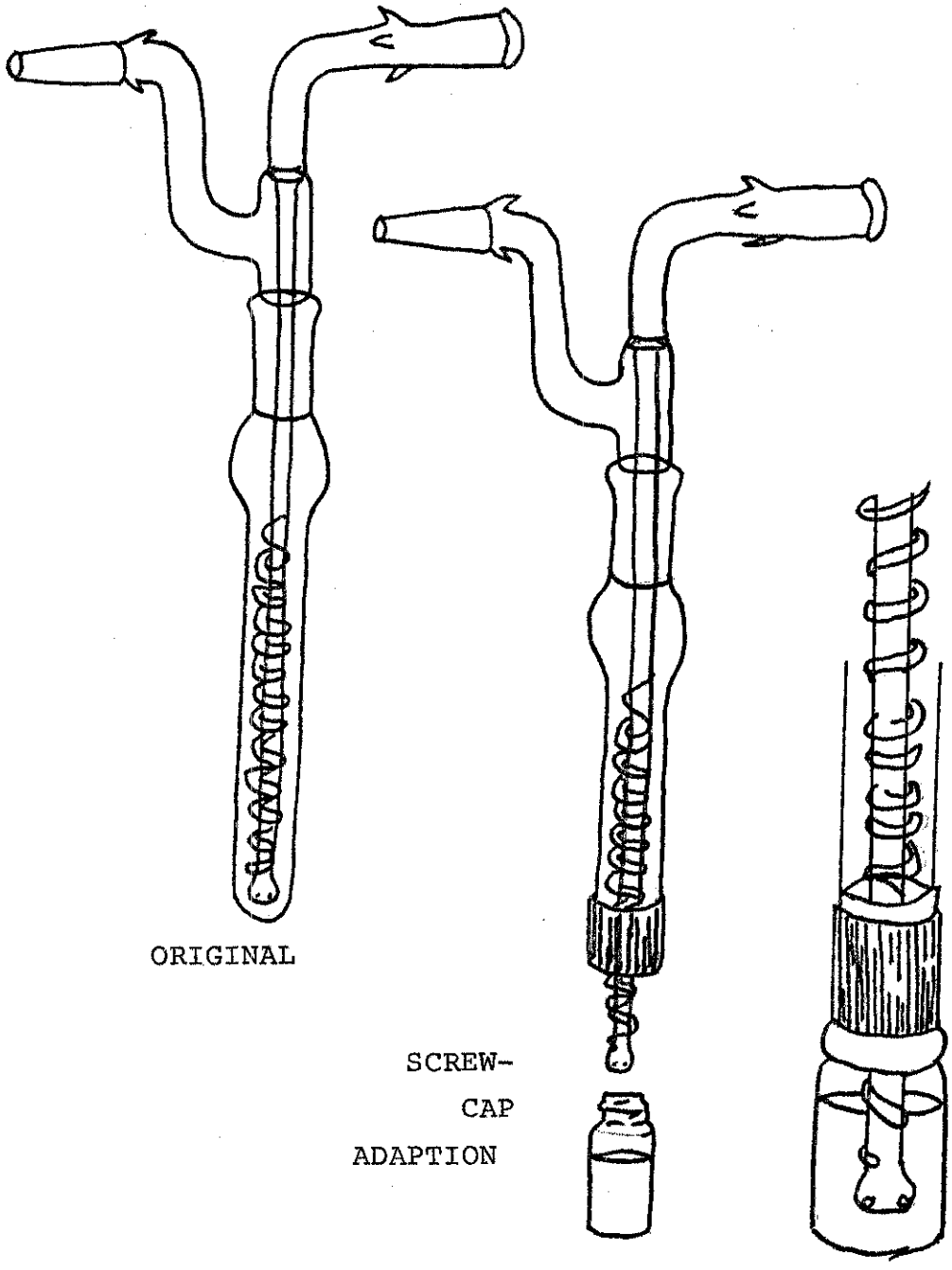


Figure 8. Adaptation of Harvey-Oxidizer Trap to scintillation vials.

V = volume of chamber (ℓ)-volume of plate (ℓ)

$^{14}C_i$ = initial ^{14}C (cpm) injected into chambers

A = colonized area of plate (m^2)

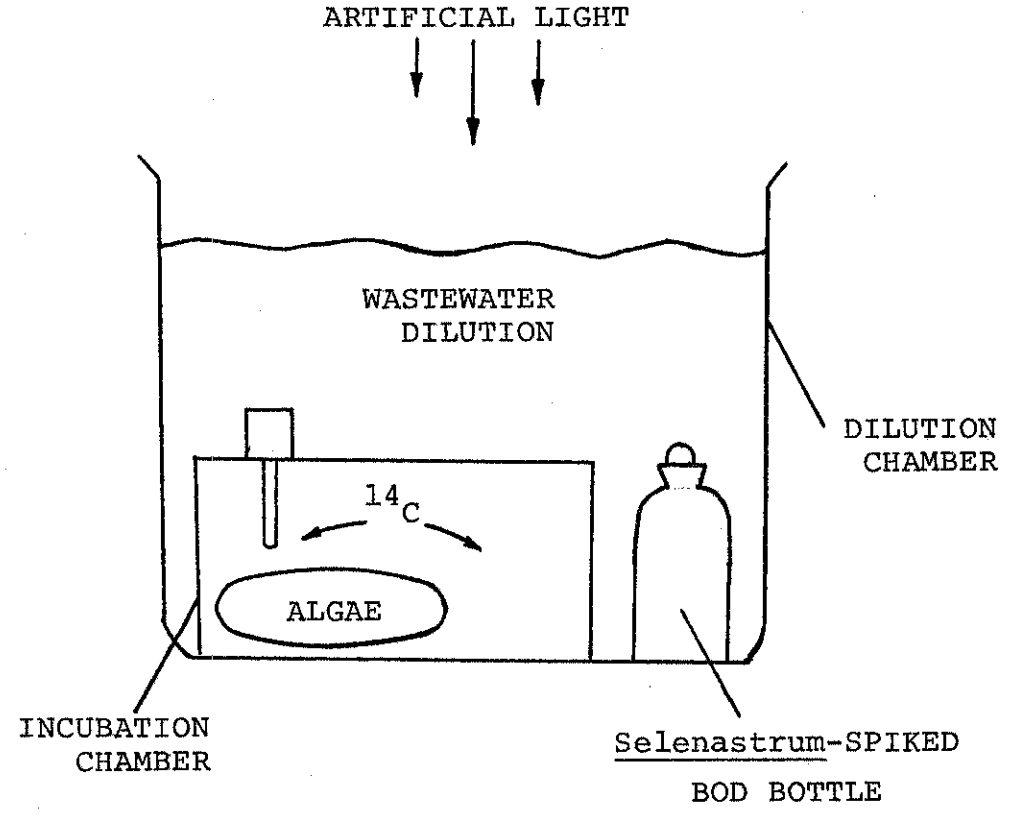
T = incubation time (hr)

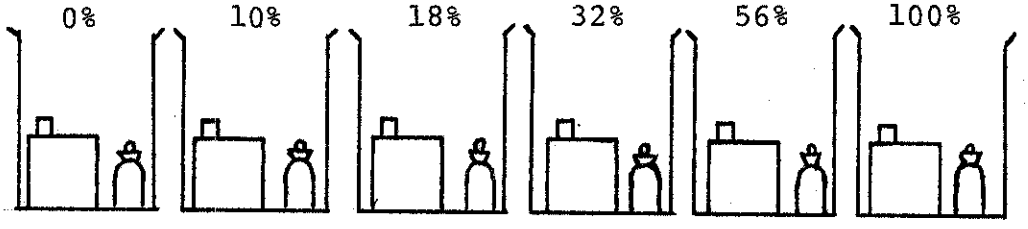
1.064 = isotopic correction factor for ^{14}C (Standard Methods, 14th edition)

^{14}C Color Bioassay

In order to determine the effect of pulp mill effluent on photosynthesis, a bioassay was designed to remove the test organism from potential chemical toxicants but still subject it to the light-attenuating properties of the darkly stained wastewater (Dickson and Rodgers, 1980). Figure 9 shows a diagram of the bioassay design.

Dilution series.--At each quarterly survey, wastewater from the Texarkana mill's finished effluent lagoon was diluted with Station 2, Sulphur River water for a series of waste concentrations. The series included static 100, 56, 32, 18, 10 and zero per cent (v/v) waste effluent concentrations. Three, eight-liter replicates of each concentration were each contained in twelve liter capacity plastic tubs. The dilutions, eighteen in all, were exposed to artificial light to effect photosynthetic response in algal





EXAMPLE DILUTION SERIES (%BKME v/v)

Figure 9. Laboratory light-attenuation (color) bioassay design (from Dickson and Rodgers,1980).

test organisms described below. Two additional zero per cent wastewater dilutions were used for incubation of test algae in the dark.

Physical parameters.--The light source for the bioassay was a series of fourteen 40-W Cool-White fluorescent light bulbs. A dilution chamber containing eight liters of distilled water was used to identify eighteen positions receiving 400 to 500 ft-c at the water's surface. These light measurements were taken with the Protomatic submarine photometer. Each replicate waste concentration was then randomly assigned a permanent position beneath the light banks. All incubations throughout the study were at room temperature (23° to 26° C).

Test organisms.--Selenastrum capricornutum Printz. and Station 2, Sulphur River periphyton were assayed for ^{14}C-assimilation in the bioassays described. Selenastrum was chosen as a control algal species to allow comparison of individual survey results and as a reference organism whose photosynthetic capabilities under controlled conditions are well-documented (EPA, 1979). Selenastrum was maintained in Bold's modification of Bristol's Medium (Bold, 1949) in two liter stock quantities. Growth conditions were room temperature and incident light from a north window. The periphyton attached to ceramic plates

were replicates of those communities assayed *in situ*, upstream of the mill's discharge. The periphyton were brought back on ice to the laboratory. It was proposed that by using naturally-occurring algal populations from the Sulphur River, photosynthetic rates as affected by BKME color might be predicted from bioassay results, given known rates of river and mill discharge flows.

Procedure.--Eighteen light and two dark 300-mℓ Wheaton bottles were filled with Station 2 river water and spiked with *Selenastrum* to a final concentration of 1000 cells·$m\ell^{-1}$. Eighteen light and two dark periphyton chambers (as used for *in situ* experiments) were also filled with Station 2 river water, and a replicate, artificial substrate with attached periphyton was placed in each. River water used to fill bottles and chambers was passed through 1.5 x 1.5 mm mesh screen to remove duckweed, conglomerations of filamentous algae, and other large particulates. One mℓ of $NaH^{14}CO_3$ solution (ten μCuries $m\ell^{-1}$) was injected into each bottle. Two mℓs of the ^{14}C-labelled bicarbonate solution were injected into the chambers. One bottle and one periphyton chamber were then placed into each replicate dilution and allowed to incubate four hours. One mℓ of 3N H_2SO_4 per *Selenastrum* bottle and five mℓs on each periphyton sample were used to

stop photosynthesis. The extent of ^{14}C-assimilation by Selenastrum was determined as described previously for phytoplankton. Periphyton productivity was assayed by the oxidation method in use at the time the bioassay was performed.

After the incubation period, light readings and water chemistry samples were taken for each replicate waste dilution. Surface and five cm-deep readings for total incident light intensity were made with the Protomatic submarine photometer. Light attenuation measurements were also taken with the IL 300 Research Radiometer for each of its ten wavelength ranges. Radiometer readings were made inside a periphyton chamber filled with Station 2 river water and submerged in each exposure chamber. Extinction coefficients were calculated as previously described. Water samples were analyzed for true and apparent color, turbidity, total suspended and dissolved solids, and total and dissolved organic carbon.

Standard Incubation - Phytoplankton Assay

Water samples, with their respective photoplankton populations, were collected at Stations 2, 2NT, 3L, 3R, and 4, and returned on ice to the laboratory. Triplicate 300-mℓ Wheaton light and dark bottles were filled for each station and allowed to equilibrate to 20° C. The

bottles were injected with one mℓ of ^{14}C-labelled bicarbonate solution (approximately ten μCuries·mℓ$^{-1}$) and placed in a Percival growth chamber. The assay incubated for four hours in growth conditions of 22 ± 1°C and 400 ft-c light intensity furnished by four, 40 W, Cool-White fluorescent lights. Addition of one mℓ of 3N H_2SO_4 to each bottle terminated the assay. The extent of ^{14}C-assimilation was determined as previously described for phytoplankton. This assay represented a control procedure for *in situ* phytoplankton productivity experiments. By providing standard incubation conditions for each sample, rate variations resulting from differences in *in situ* physical growth parameters would be reduced. Therefore, any significant differences between stations might be attributed to variation in water chemistry or biomass and better indicate a potential impact from BKME.

Data Analysis

A National Advanced System (NAS) 5000 computer was used for analysis of data. The Statistical Analysis System (SAS) (Helwig and Council, 1979) and MUSIC (IBM, 1981) interactive programs were used to perform all calculations, non-parametric analyses of variance and correlation, and probit analyses. The statistical tables in Zar (1974) were consulted in tests for statistical significance.

CHAPTER III

RESULTS

July 1980 Experiments

The July 1980 quarterly survey was only a partial success. In situ operations were efficiently performed. An error in sample preservation, however, resulted in the loss of field samples. Therefore, no data for this survey are presented.

Despite this loss of information, the first survey experience did provide an opportunity to review and test the efficacy of field and laboratory methods. Changes in procedures made after this survey proved to be beneficial for the remainder of the study. As previously mentioned, artificial substrate orientation was changed from horizontal to vertical. Problems with suitable sample preservation and handling caused the periphyton ATP assay to be discontinued. The first color bioassay was performed; however, the samples were sacrificed to establish precision and efficiencies for phytoplankton and periphyton ^{14}C-recovery methods. For example, subsampling precision, reaction time and temperature, sample-to-acid ratios, and trapping volumes were determined for the wet-oxidation technique at this time.

River and Wastewater Flows

The seasonal variation in the lower Sulphur River flow is indicated in Table III. The extremes in flow levels encountered during survey months ranged from 0.28 $m^3 \cdot sec^{-1}$ in January and April, 1981 to 285 $m^3 \cdot sec^{-1}$ in July 1981. Although the magnitude of river flow extremes was not unexpected, the absence of mill discharge through most of the study was unforeseen (Table III).

The lack of waste effluent was a consequence of unusual climatic conditions and compliance with state discharge regulations. A severe drought in the summer of 1980 kept Wright Patman Lake levels below minimum, which curtailed dam releases during the subsequent winter. Normally, winter months are periods of high river flow when the mill discharges substantial amounts of its treated waste. Permitted waste discharge volumes cannot exceed 16.2 percent of Sulphur River flows (TDWR permit #01339). River flow below 28.5 $m^3 \cdot sec^{-1}$ is generally not conducive to waste discharge because of the increased potential for violation of prescribed maximum water chemistry levels downstream. In July 1981, unseasonal rainfall brought flooding to the area and maximum discharge from Wright Patman. However, the mill was still unable to release its waste. Dissolved oxygen (DO) levels in the river were at or below 4.0 $mg \cdot \ell^{-1}$, and the mill's

TABLE III

WRIGHT PATMAN DAM AND THE IP TEXARKANA MILL DISCHARGE LEVELS ($m^3 \cdot sec^{-1}$) TO THE LOWER SULPHUR RIVER DURING QUARTERLY SURVEYS. WASTE EFFLUENT EXPOSURE HISTORY FOR DOWNSTREAM ARTIFICIAL SUBSTRATES.

Survey Month	Wright Patman Flow	Mill Effluent	Percent Dilution	Incubation Period (Days)	Waste Exposure Period (Days)
Oct 80	28.50	0	0	20	0
Jan 81	.29	0	0	28	9*
Apr 81	.29	0	0	29	22**
Jul 81	285.00	0	0	28	0
Oct 81	3.96	1.6	29	22	22

* Mill discharge stopped 11 days before survey date

** Mill discharge stopped 7 days before survey date

discharge permit prevented discharge if river DO levels cannot be maintained at or above 5.0 $mg \cdot \ell^{-1}$ (TDWR #01339). In August 1981, International Paper was granted their request for a variance on their permit and began releasing in September. The BKME discharge level during the October 1981 survey (Table III) represented approximately 41 per cent of the river flow or a 29 percent (v/v) BKME concentration downstream. The extent of BKME impact on primary productivity during this study, therefore, could only be assessed from the results of the October 1981 monitoring. However, data from non-discharge surveys represented normal variation of river characteristics between stations and were useful for general comparisons with October 1981 results. As a result of the abnormally high waste discharge, the October 1981 survey results were assumed to represent a worst-case situation.

Table III also indicates the incubation history for artificial substrates prior to and including each survey date. Downstream artificial substrates and their attached communities were exposed to BKME prior to the January and April 1981 survey dates; however, mill discharge ceased at least one week before each sampling date. Therefore, January and April 1981 substrates were not considered as representing periphyton communities influenced by BKME. This conclusion was based on the following assumptions.

1. One week was sufficient time to purge the physical-chemical regime of a BKME environment and replace it with one representing upstream conditions. Water chemistry and light attenuation data for these surveys (Appendix A) suggested this assumption was valid.
2. Periphyton turnover rates were rapid enough to significantly change the community to reflect upstream conditions (Patrick, 1971).

Total Irradiance

Variations in solar irradiance between surveys are shown in Table IV. These values were measured with a Solar Monitor LI-200SB. The wavelength range from 300 to 800 nm is reported as defining the quantity of light energy available to the various photosynthetically available radiation (PAR) (Vollenweider, 1974). The LI-200SB measures the light energy integrated for the 400 to 1100 nm range and was assumed to approximate the PAR to the Sulphur River system.

Fifty per cent of the above values were taken as the amount of incident radiation that occurred during respective 1000-1400 hr incubation periods (Rodgers, unpublished thesis, 1974).

TABLE IV

SOLAR IRRADIANCE DURING ON-SITE PRODUCTIVITY EXPERIMENTS

Survey	Total Daily Irradiance ($W \cdot m^{-2}$) 400 - 1100 nm	PAR During *in situ* Incubation
Oct 80	4791	2395
Jan 81	1513	756
Apr 81	4374	2187
Jul 81	5940	2970
Oct 81	2082	1041

Field Experiments

Periphyton

Periphyton productivity, chlorophyll *a*, ash-free dry weight, PE, and structural index were calculated for each survey at each river station (Appendix B). Figures 10 - 14 illustrate the seasonal and between station differences for each periphyton parameter, respectively. Non-parametric analysis of variance for each parameter during the October 1981 survey is summarized in Table V. The results show no significant differences between upstream and downstream stations for any of the parameters. Kruskal-Wallis statistics indicate significant differences for ash-free dry weight and structural index ($\alpha = .05$). However, non-parametric, multiple range tests do not show these differences to exist between the reference and experimental sites. Statistical analysis results for non-discharge periphyton productivity are summarized in Appendix C.

In situ rates and ash-free dry weight were highest at Station 3R. PE and chlorophyll *a* concentration were greatest at Stations 2 and 3L, respectively. Values of all periphyton parameters were lowest at Station 4. Compared with the upstream stations, Station 4 productivity rates were only 44 and 36 per cent of Station 2 and 2NT rates, respectively.

Phytoplankton

Phytoplankton parameters -- primary productivity, chlorophyll a, and PE -- were calculated for each survey at each river station (Appendix B). The seasonal and between station differences for each of the above phytoplankton parameters are indicated in Figures 15 - 18. Table VI summarizes analysis of variance results for each parameter during mill discharge. No difference between reference and experimental sites was found for planktonic chlorophyll a. However, upstream stations were shown to be significantly different from downstream Stations 3R and 4 for productivity rate and PE. Non-discharge survey statistical results are given in Appendix C.

A steady decrease in in situ rates from upstream to downstream stations was noted (Table VI). Station 4 rates were found to be only 36 and 39 per cent as fast as those at Stations 2 and 2NT, respectively. Average PE ratios and chlorophyll a concentrations were also higher at upstream stations.

Water Quality and Environmental Parameters

The influence of BKME on water quality parameters during the October 1981 survey is indicated in Table VII. Substantial increases in almost every parameter were

noted at Stations 3L and 3R relative to upstream stations. Station 4 data show a subsequent reduction of these high concentrations to near-upstream levels.

Figures 19 and 20 show the light-absorbing character of BKME determined from on-site spectroradiometer measurements and laboratory spectrophotometric analysis, respectively (October 1981 survey). Strong light attenuation from absorption was found in the shorter wavelength regions of the PAR spectrum.

Figure 21 illustrates the increased light attenuation at downstream sites during mill discharge. Here also, the ultra-violet to blue portion of the spectrum was strongly absorbed, particularly at Station 4.

Total PAR attenuation measured during each survey, at each station and in wastewater is shown in Figure 22. These coefficients were calculated from the Protomatic photometer readings. These data indicate a farily consistent light-absorption capacity of the BKME, while river values are more variable.

Laboratory Experiments

Color Bioassay

Significant decreases in bioassay productivity rates with increasing BKME concentration were found for both Sulphur River periphyton and Selenastrum capricornulum

Prinz. (Figures 23 and 24, respectively). The values shown in Figures 23 and 24 are the total mean rates for the pooled results of the five runs. These data indicate that productivity rates measured in 100 per cent wastewater concentrations were approximately three orders of magnitude less than those measured in the zero per cent concentrations.

The reduction in total PAR through the bioassay dilutions is illustrated in Figure 25 for the individual bioassay runs. The data in this figure suggest the river water accounted for most of the variation in light regime between runs (note zero per cent wastewater concentration). Figure 26 presents a representative example of the spectral distributions of light absorbed by each BKME concentration (October 1981). Attenuation was strongest in the blue region, similar to results shown in previous extinction coefficient histograms for field data. Results of water quality analyses on wastewater dilutions for the July and October 1981 runs are on file.

Standard Incubation

The October 1981 results of the laboratory ^{14}C incubation of indigenous Sulphur River phytoplankton are included in Table VI for comparison with *in situ* rates. Average rates decreased downstream from Station 2. However, the Newman-Keuls grouping of ranked sums does not

clearly indicate significant differences existing between upstream and downstream stations. Non-discharge survey results and statistical analyses are given in Appendices B and C, respectively. Figure 18 illustrates the results of the standard incubation assay for the entire study.

Correlation of Productivity to Environmental Parameters

Field

Tables VIII and IX are correlation matrices, presenting Spearmans' rank correlation coefficients between productivity parameters and physical-chemical variables. Table VIII identifies which, if any, light attenuation coefficients may account for the observed variations in productivity. All significant correlations with light indicated inverse relationships except for periphyton structural index. The data suggest this was a result of variations in ash-free dry weights rather than chlorophyll *a* concentrations. In general, phytoplankton *in situ* rates showed the highest correlation with light measurements ($r = .832$, significance = .0001 with total PAR extinction). Variations in current velocity apparently had no significant relationship to periphyton structure.

Significant correlations of productivity with selected water chemistry are shown in Table IX. Highest

correlations were found for ash-free dry weight with turbidity and dissolved organic carbon (DOC) (r = -.853 and .920, respectively). Phytoplankton productivity rates were negatively correlated with color, total dissolved solids (TDS), and total organic carbon (TOC).

Laboratory

Productivity rates of *Selenastrum* and Sulphur River periphyton measured in the bioassay were tested for correlation with light extinction and water chemistry data (Table X). Significant correlations (α = .05) were found between the rates and all chemical variables, with the exception of turbidity. Productivity rates were correlated with all light extinction data. Highest correlation coefficients were found with the attenuation of 495-505 nm, 555-565 nm ranges, and total PAR. The extinction of the 356-365 nm range was the least correlated with productivity.

Model Prediction of *In Situ* Productivity

As shown in Figures 27 and 28, probit analysis on pooled data predicts ED_{50} values of 20 and 21 per cent BKME concentration for Sulphur River periphyton and *Selenastrum*, respectively. The slope for both probability plots is 0.044.

The predicted reduction in photosynthesis from these plots for a 29 per cent BKME concentration is approximately

65 per cent. The observed in situ reductions in productivity from Stations 2 and 2NT to Station 4 was roughly 60 per cent for both periphyton and phytoplankton. Chi-square analysis to statistically compare the observed and predicted results could not be done since data for only one such comparison were available.

TABLE V.

PERIPHYTON PRODUCTIVITY RESULTS FOR THE OCTOBER 1981 SURVEY. STATION VALUES ARE MEANS OF THREE REPLICATES. ANALYSIS OF VARIANCE BETWEEN STATIONS - COEFFICIENT OF VARIATION (C.V.), KRUSKAL WALLIS SIGNIFICANCE LEVEL, AND NEWMAN-KEULS MULTIPLE RANGE TEST GROUPS. STATIONS GROUPED TOGETHER ARE NOT SIGNIFICANTLY DIFFERENT AT α = .05.

Parameter Units	Station					C.V.	Kruskal Wallis	Newman-Keuls Groups
	2	2NT	3L	3R	4			
In Situ Rates mg $C \cdot m^{-2} \cdot hr^{-1}$	2.74	3.39	3.16	4.08	1.21	55.3	NS	2NT 3R 2 3L 4
^{PE} mg $C \cdot h^{-1}$/mg chla	16.49	11.82	8.73	15.95	5.18	71.5	NS	2 3R 3L 2NT 4
Chlorophyll a mg chla$\cdot m^{-2}$	0.26	0.41	0.34	0.27	0.24	54.0	NS	3L 2NT 3R 4 2
Ash-Free Dry Weight mg org$\cdot m^{-2}$ ($x10^3$)	21.37	5.81	26.86	144.58	3.83	120.4	.025	3R 3L 2 2NT 4
Structural Index mg chla/mg org ($x10^{-4}$)	0.12	0.71	0.13	0.02	0.62	68.5	.05	2NT 4 3L 2 3R

NS - not significant at α= .05

TABLE VI.

PHYTOPLANKTON PRODUCTIVITY RESULTS FOR THE OCTOBER 1981 SURVEY. STATION VALUES ARE MEANS OF THREE REPLICATES. ANALYSIS OF VARIANCE BETWEEN STATIONS - COEFFICIENT OF VARIATION (C.V.), KRUSKAL WALLIS SIGNIFICANCE LEVEL, AND NEWMAN-KEULS MULTIPLE RANGE TEST GROUPS. STATIONS GROUPED TOGETHER ARE NOT SIGNIFICANTLY DIFFERENT AT $\alpha = .05$.

Parameter Units	Station					C.V.	Kruskal Wallis	Newman-Keuls Groups
	2	2NT	3L	3R	4			
In situ Rates mg $C \cdot m^{-3} \cdot hr^{-1}$	444.2	411.2	252.1	188.7	160.9	16.5	.025	2 2NT 3L 3R 4
PE mg $C \cdot hr^{-1}$/mg chla ($\times 10^3$)	34.94	21.52	22.52	14.57	15.32	19.2	.025	2 3L 2NT 4 3R
Chlorophyll a mg chla$\cdot m^{-2}$	0.013	0.019	0.011	0.014	0.011	26.0	NS	2NT 2 3R 3L 4
Standard Incub. rates mg $C \cdot m^{-3} \cdot hr^{-1}$	185.7	163.8	127.9	122.2	77.4	14.3	.025	2 2NT 3R 3L 4

NS - not significant at $\alpha = .05$.

TABLE VII

BIOLOGICAL, PHYSICAL, AND CHEMICAL DATA COLLECTED DURING THE OCTOBER 1981 SURVEY. MEAN VALUES OF THREE REPLICATES

	Periphyton					Phytoplankton			
Sta.	In Situ ^{14}C	PE	Chl *a*	AFDRYWT ($\times 10^3$)	S.I. ($\times 10^3$)	In Situ ^{14}C	PE	Chl *a*	Std. incub. ^{14}C
2	2.73600	16.49	0.256333	21.37	0.12	444.190	34.94	0.0130000	185.68
2NT	3.39333	11.82	0.413000	5.81	0.71	411.197	21.52	0.0190000	163.82
3L	3.16100	8.73	0.340667	26.86	0.13	252.117	22.52	0.0113333	127.85
3R	4.07767	15.95	0.277333	144.6	0.02	188.663	14.57	0.0143333	122.16
4	1.20867	5.18	0.238333	3.84	0.62	160.913	15.32	0.0106667	77.36

Extinction coefficients (ε) for wavelength (λ) ranges.

	344-356	395-405	445-455	495-505	545-555	595-605	645-655	695-705	745-755	795-805
2	6.817	10.215	7.639	5.537	3.964	3.282	4.458	3.173	3.861	4.543
2NT	7.686	8.104	10.173	10.060	10.823	10.929	11.025	10.787	10.504	10.560
3L	9.042	16.129	17.762	10.040	10.858	10.504	7.974	4.342	6.645	5.817
3R	8.757	6.784	12.642	10.050	10.504	10.571	7.547	8.969	6.662	5.924
4	11.765	24.752	21.978	10.225	11.779	12.005	10.395	8.137	7.974	6.873

TABLE VII--Continued.

Sta.	°C	pH	Conductivity (µS)	DO (mg/ℓ)	Current Velocity (mm/min)	Surface Up-Welling	Surface Down-Welling	1 Meter Up- ($W \cdot m^2$)	1 Meter Down- ($W \cdot m^2$)	ε Total PAR
2	23	6.95	200	4.6	14.6000	80	8.8	2.34667	0.300	3.52943
2NT	24	6.96	200	4.6	9.6333	72	7.2	1.68000	0.212	3.75799
3L	24	7.06	700	4.7	2.1333	92	2.2	0.01040	0.002	9.09091
3R	23	7.07	700	4.7	5.2000	100	2.0	0.01240	0.002	8.99281
4	24	6.92	250	4.6	12.5333	92	1.8	0.00440	0.002	9.95025

	Acidity mg/ℓ $CaCO_3$	Alkalinity mg/ℓ $CaCO_3$	Hardness mg/ℓ $CaCO_3$	Turbidity NTV	TDS mg/ℓ	TSS mg/ℓ	TOC mg/ℓ	DOC mg/ℓ	BOD mg/ℓ
2	6	76.6667	74.667	19.0000	147.667	31.0000	9.0000	6.0000	2.93333
2NT	4	75.0000	77.333	19.6667	150.667	36.0000	7.3333	5.6667	3.16667
3L	7	90.0000	133.333	17.0000	371.333	26.6667	16.0000	13.0000	4.06667
3R	6	90.0000	133.333	17.0000	368.500	37.6667	19.0000	16.0000	4.53333
4	5	75.0000	85.333	35.6667	184.667	74.3333	10.0000	5.0000	3.36667

TABLE VII--Continued.

Sta	Cl^- mg/ℓ	SO_4^{-2} mg/ℓ	NH_4^+ mg/ℓ	NO_3^- mg/ℓ	Ortho PO_4^{-3} mg/ℓ	Total PO_4^{-3} mg/ℓ	Apparent Color C.U.*	True Color C.U.*
2	13.000	9.0000	0.0	0.100000	0.113333	0.156333	55	30
2NT	13.000	10.0000	0.0	0.100000	0.076667	0.112333	55	30
3L	129.333	58.0000	0.2	0.400000	0.122000	0.168333	225	175
3R	119.333	53.0000	0.0	0.400000	0.114000	0.162000	225	175
4	25.000	15.6667	0.0	0.166667	0.110333	0.180667	100	60

* Chloroplatinate color units

TABLE VIII

CORRELATION RESULTS OF PRODUCTIVITY PARAMETERS WITH LIGHT EXTINCTION COEFFICIENTS, ε. SPEARMAN RANK COEFFICIENTS AND SIGNIFICANCE LEVELS FOR THE OCTOBER 1981 SURVEY (N = 15).

	Periphyton					Phytoplankton		
λ range (nm)	In Situ ^{14}C	PE	Chl *a*	Ash-free dry wt.	Struct. Index	In Situ ^{14}C	PE	Chl *a*
344-356	-0.43644 0.1039	-0.46917 0.0777	0.07638 0.7867	-0.29459 0.2865	0.29459 0.2865	-0.82923 0.0001	-0.63283 0.0113	-0.43449 0.1056
395-405	-0.48008 0.0701	-0.46917 0.0777	-0.07638 0.7867	-0.53463 0.0400	0.55646 0.0312	-0.28368 0.3055	0.04364 0.8773	-0.42349 0.1157
445-455	-0.43644 0.1039	-0.46917 0.0777	0.07638 0.7867	-0.29459 0.2865	0.29459 0.2865	-0.82923 0.0001	-0.63283 0.0113	-0.43449 0.1056
495-505	-0.39279 0.1475	-0.40370 0.1356	-0.03273 0.9078	-0.66556 0.0068	0.62192 0.0133	-0.56737 0.0274	-0.72012 0.0025	0.03850 0.8916
545-555	-0.43644 0.1039	-0.53463 0.0400	0.12002 0.6701	-0.55646 0.0312	0.62192 0.0133	-0.58919 0.0208	-0.46917 0.0777	-0.25849 0.3523
595-605	-0.39279 0.1475	-0.40370 0.1356	-0.03273 0.9078	-0.66556 0.0068	0.62192 0.0133	-0.56737 0.0274	-0.72012 0.0025	0.03850 0.8916
645-655	-0.18549 0.5081	-0.34915 0.2021	0.17457 0.5338	-0.64374 0.0096	0.75285 0.0012	-0.14184 0.6141	-0.34915 0.2021	0.24749 0.3738
695-705	0.06547 0.8167	-0.03273 0.9078	0.14184 0.6141	-0.20731 0.4585	0.22913 0.4114	-0.17457 0.5338	-0.55646 0.0312	0.40149 0.1380
745-755	-0.16366 0.5600	-0.25095 0.3670	0.07638 0.7867	-0.56737 0.0274	0.58919 0.0208	-0.25095 0.3670	-0.55646 0.0312	0.30799 0.2641
795-805	-0.16366 0.5600	-0.25095 0.3670	0.07638 0.7867	-0.56737 0.0274	0.58919 0.0208	-0.25095 0.3670	-0.55646 0.0312	0.30799 0.2641
Total PAR	-0.43523 0.1049	-0.44792 0.0941	0.05078 0.8574	-0.30647 0.2666	0.28290 0.3069	-0.83237 0.0001	-0.63652 0.0107	-0.42231 0.1168

TABLE IX

CORRELATION RESULTS OF PRODUCTIVITY PARAMETERS WITH SELECTED WATER CHEMISTRY. SPEARMAN RANK COEFFICIENTS AND SIGNIFICANCE LEVELS FOR THE OCTOBER 1981 SURVEY (N = 14).

Water Quality	Periphyton					Phytoplankton		
Parameter	In Situ	PE	Chl a	Ash-free dry wt.	Struct. Index	In Situ ^{14}C	PE	Chl a
True Color	0.03298 0.9109	-0.04476 0.8792	0.16491 0.5732	0.43112 0.1238	-0.37458 0.1870	-0.65021 0.0118	-0.42641 0.1284	-0.37352 0.1883
Apparent Color	0.03298 0.9109	-0.04476 0.8792	0.16491 0.5732	0.43112 0.1238	-0.37458 0.1870	-0.65021 0.0118	-0.42641 0.1284	-0.37352 0.1883
TDS	0.13421 0.6474	-0.01320 0.9643	0.25963 0.3701	0.40704 0.1486	-0.36744 0.1962	-0.58966 0.0265	-0.34103 0.2328	-0.41750 0.1375
TSS	-0.19362 0.5072	-0.18042 0.5371	-0.20682 0.4781	-0.69747 0.0056	0.59846 0.0238	-0.41144 0.1438	-0.42684 0.1280	-0.15836 0.5887
TOC	0.05358 0.8557	0.09153 0.7557	-0.02009 0.9456	0.52908 0.0517	-0.54917 0.0420	-0.63624 0.0144	-0.36835 0.1950	-0.42249 0.1323
DOC	0.47584 0.0855	0.36428 0.2004	0.27776 0.3363	0.91980 0.0001	-0.73994 0.0025	0.07741 0.7925	0.09335 0.7509	0.07105 0.8093
Turbidity	-0.41647 0.1385	-0.32593 0.2554	-0.35988 0.2063	-0.85331 0.0001	0.68129 0.0073	-0.18786 0.5201	-0.27614 0.3393	0.03418 0.9077
BOD	0.21682 0.4565	0.13275 0.6510	0.21461 0.4613	0.59072 0.0261	-0.37390 0.1879	-0.32080 0.2634	-0.23452 0.4197	-0.17038 0.5603
Current Velocity	-0.27753 0.3367	-0.05507 0.8517	-0.38546 0.1735	-0.42511 0.1297	0.24009 0.4084	0.18062 0.5366	0.18502 0.5266	0.00111 0.9970

TABLE X

CORRELATION RESULTS OF COLOR BIOASSAY ^{14}C-PRODUCTIVITY RATES WITH LIGHT EXTINCTION, ε, AND SELECTED WATER CHEMISTRY. SPEARMAN RANK COEFFICIENTS, SIGNIFICANCE LEVELS, AND SAMPLE SIZE

Bioassay Productivity	344-356 nm	395-405 nm	445-455 nm	495-505 nm	545-555 nm	596-605 nm
Selenastrum	-0.71311	-0.94840	-0.76677	-0.98349	-0.97317	-0.95872
	0.0009	0.0001	0.0002	0.0001	0.0001	0.0001
	18	18	18	18	18	18
Sta. 2 periphyton	-0.55860	-0.84667	-0.65256	-0.87558	-0.88797	-0.86422
	0.0160	0.0001	0.0033	0.0001	0.0001	0.0001
	18	18	18	18	18	18
	645-655 nm	695-705 nm	745-755 nm	795-805 nm	Total PAR	True Color
Selenastrum	-0.95872	-0.95666	-0.90918	-0.87203	-0.97523	-0.96835
	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
	18	18	18	18	18	36
Sta. 2 periphyton	-0.84048	-0.84461	-0.77646	-0.76820	-0.86835	-0.71163
	0.0001	0.0001	0.0002	0.0002	0.0001	0.0001
	18	18	18	18	18	36
	Apparent Color	TDS	TSS	TOC	DOC	Tur-bidity
Selenastrum	-0.96854	-0.96601	0.47114	-0.60732	-0.91478	0.07382
	0.0001	0.0001	0.0037	0.0001	0.0001	0.7710
	36	36	36	36	36	18
Sta. 2 periphyton	-0.71138	-0.74960	0.49008	-0.50448	-0.52600	0.09702
	36	36	36	36	36	18

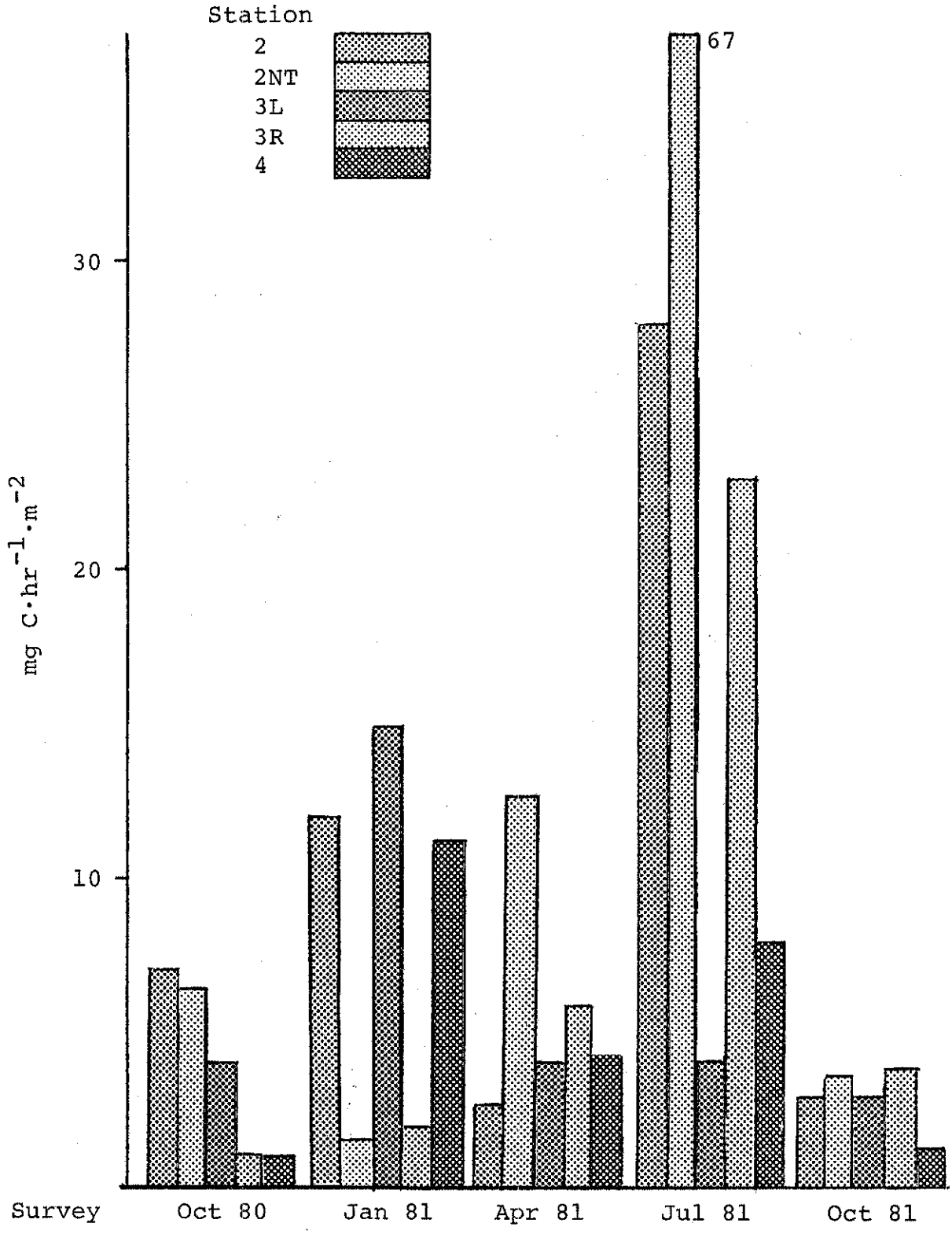


Figure 10. In situ periphyton mean productivity rates (n = 3) for each station and each survey.

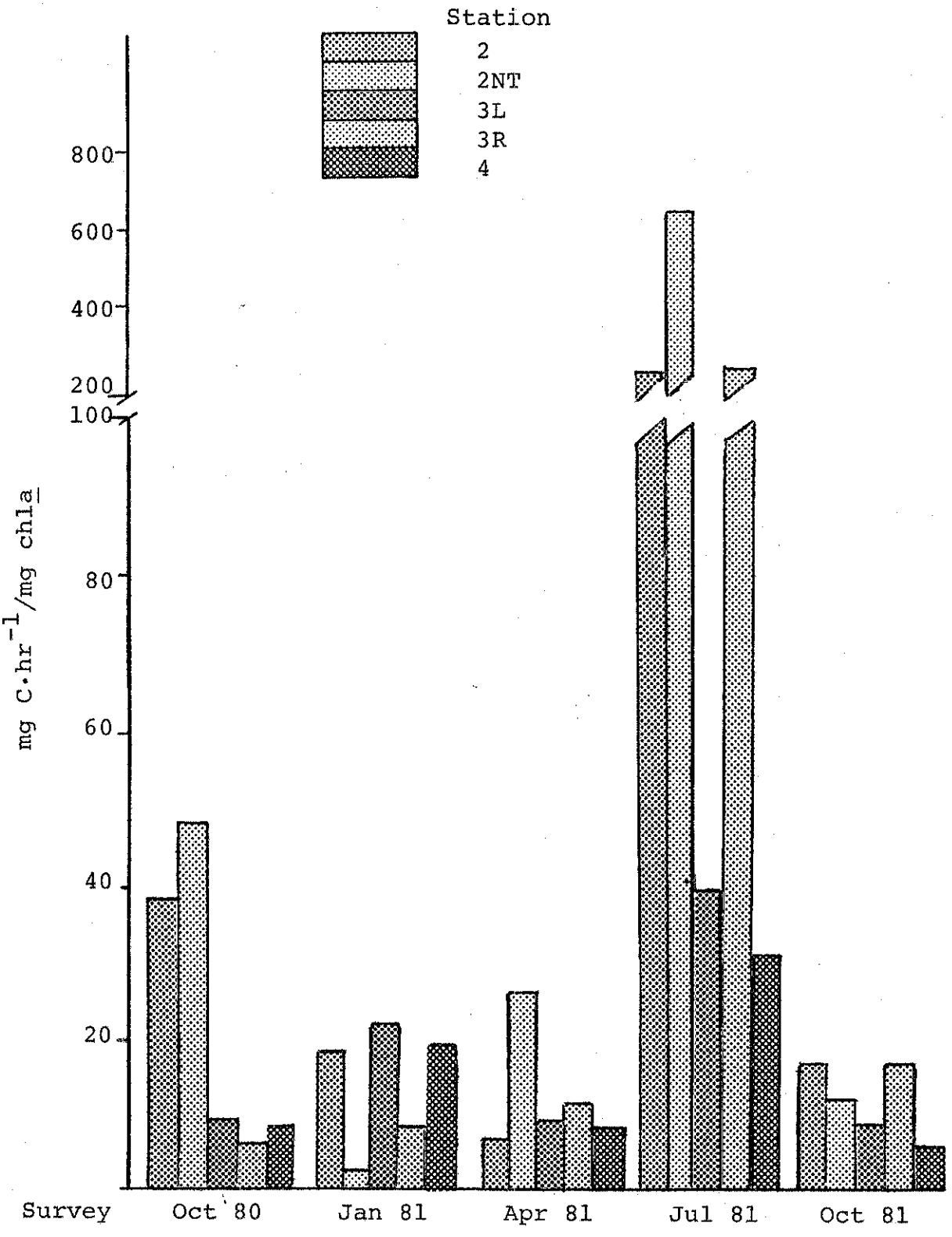


Figure 11. Periphyton productivity efficiency ratio, PE. Mean values (n = 3) for each station and each survey.

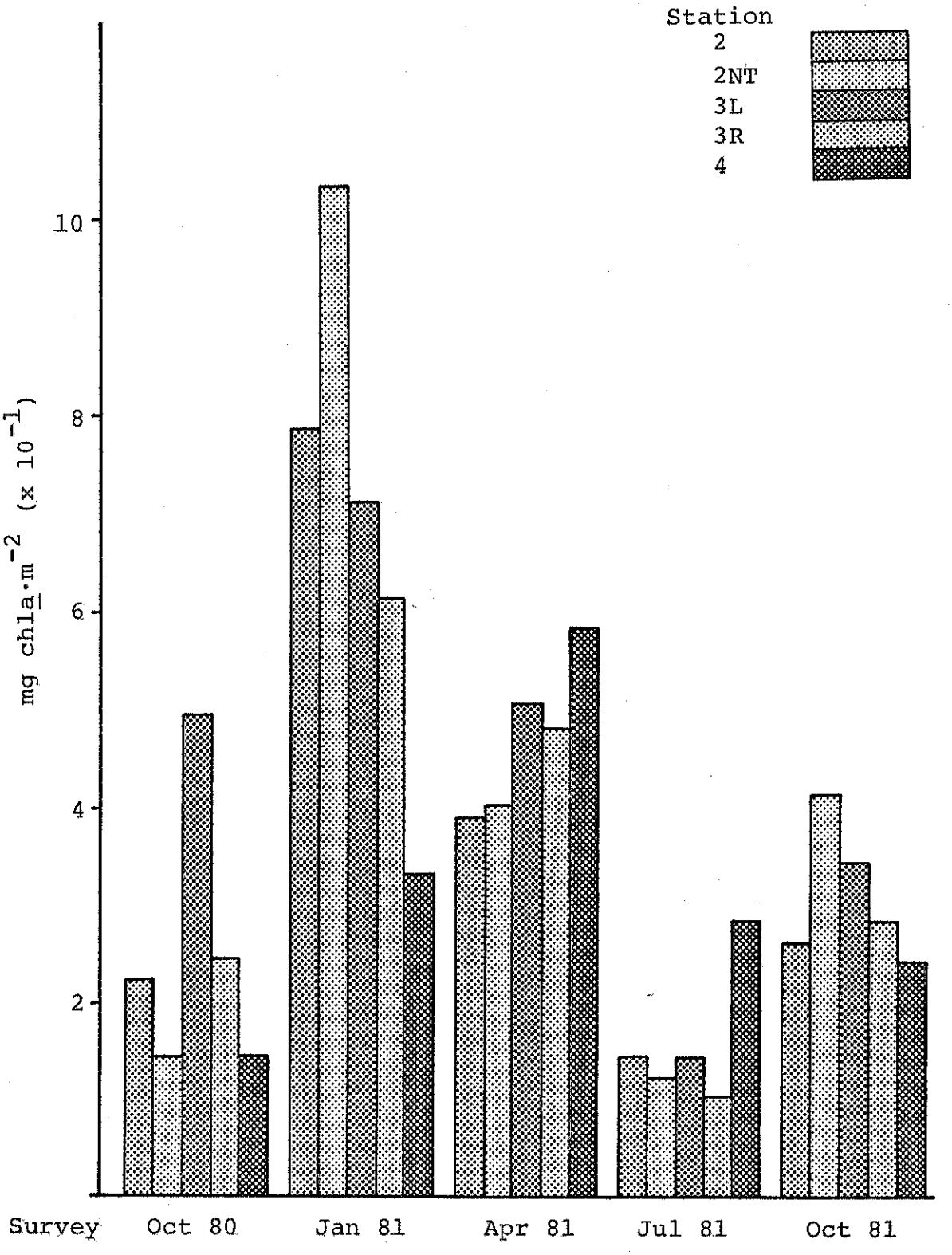


Figure 12. Periphyton chlorophyll a concentrations for each station and each survey. Mean values (n = 3).

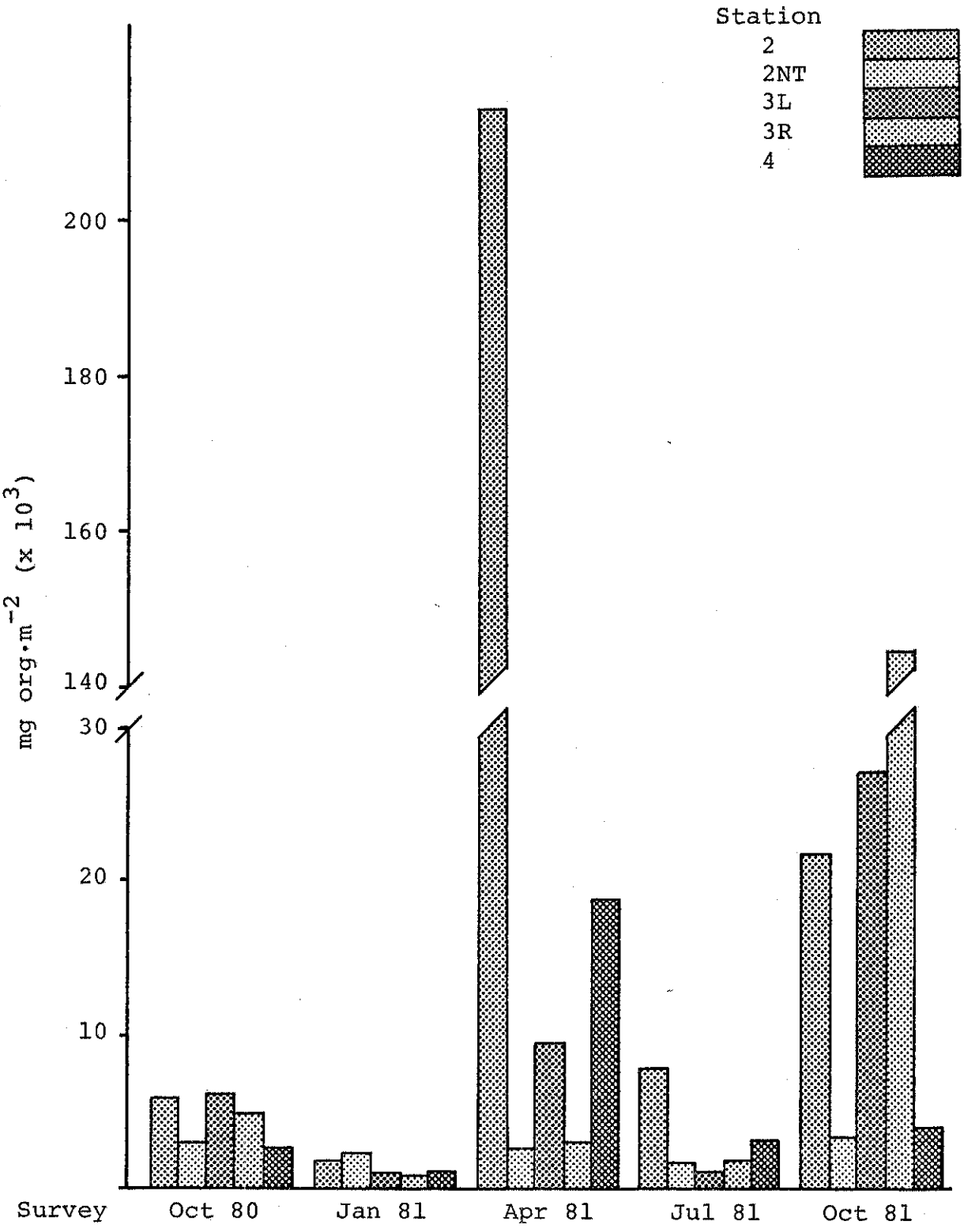


Figure 13. Periphyton ash-free dry weights for each station and each survey. Mean values (n = 3).

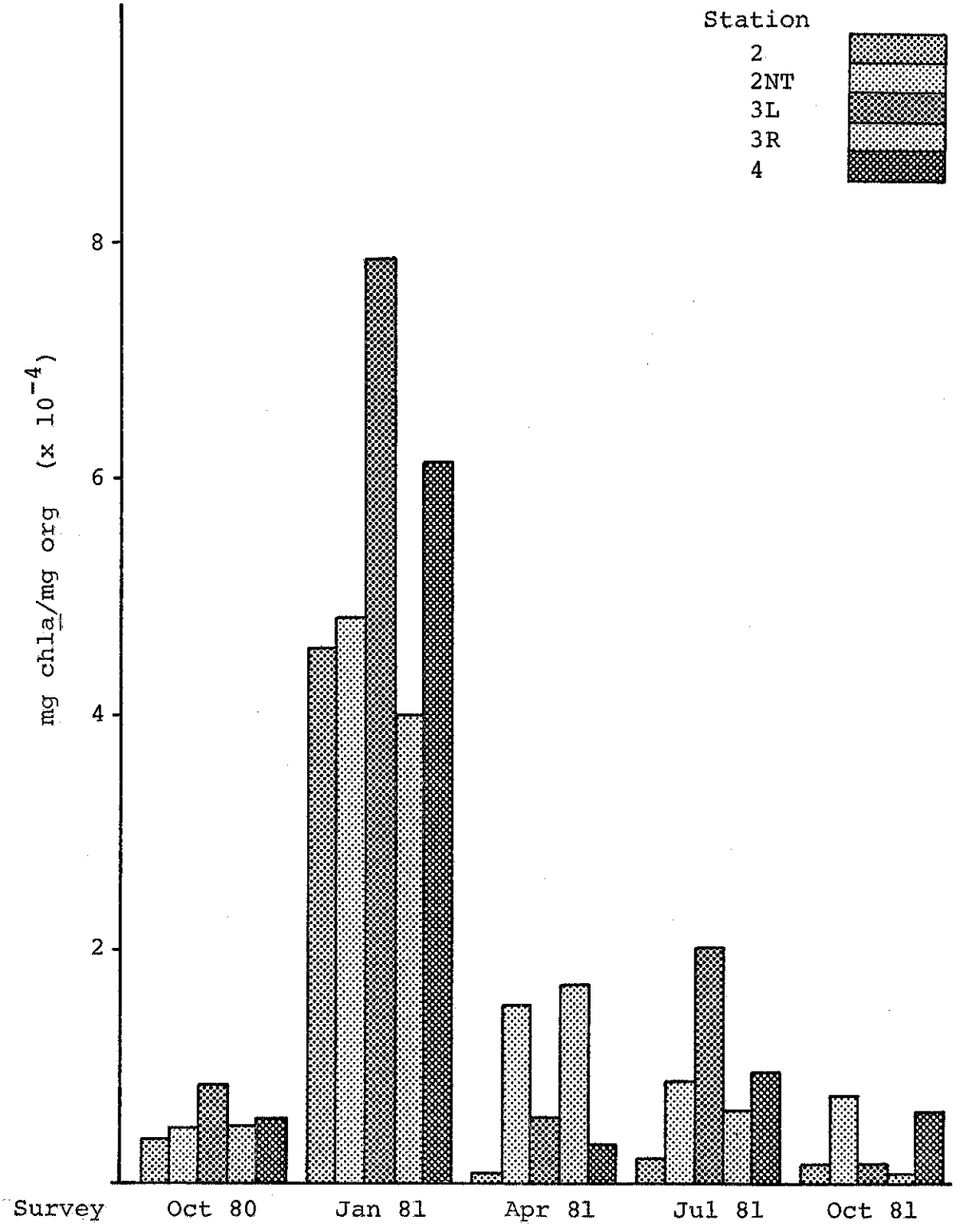


Figure 14. Periphyton structural index values for each station and each survey. Mean values (n = 3).

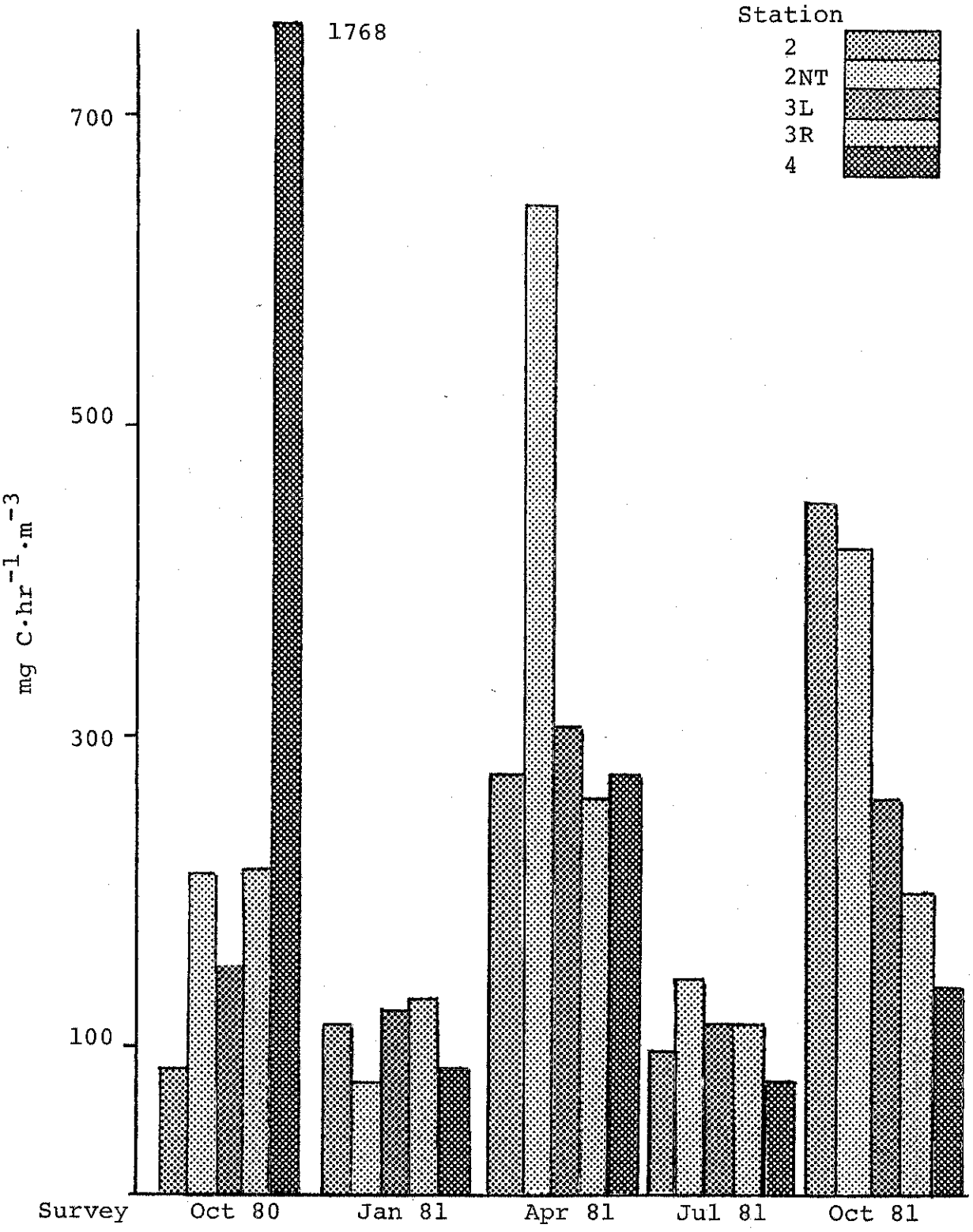


Figure 15. In situ phytoplankton, mean productivity rates (n = 3) for each station and each survey.

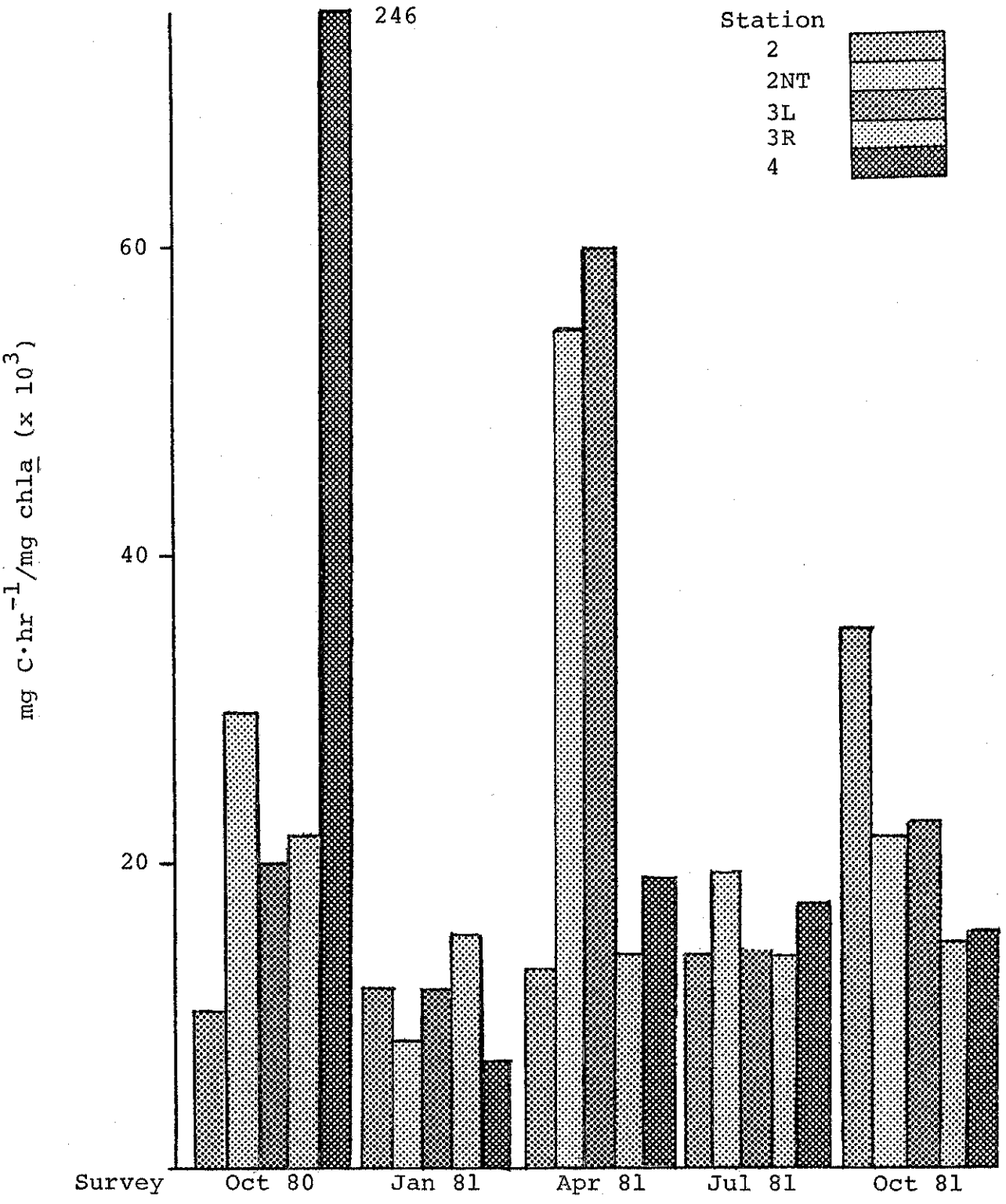


Figure 16. Phytoplankton productivity efficiency ratio, PE. Mean values (n = 3) for each station and each survey.

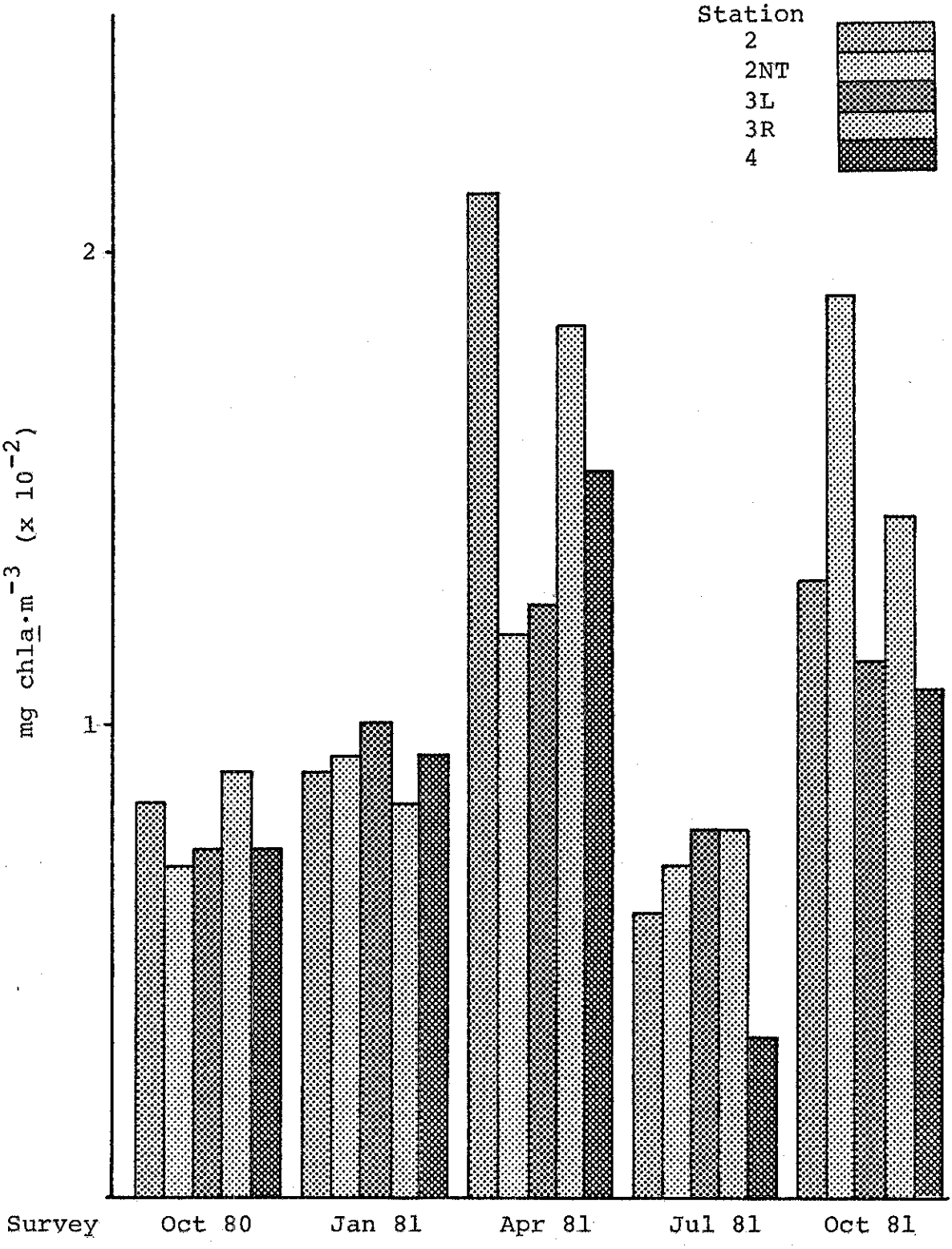


Figure 17. Planktonic chlorophyll a for each station and each survey. Mean values (n = 3).

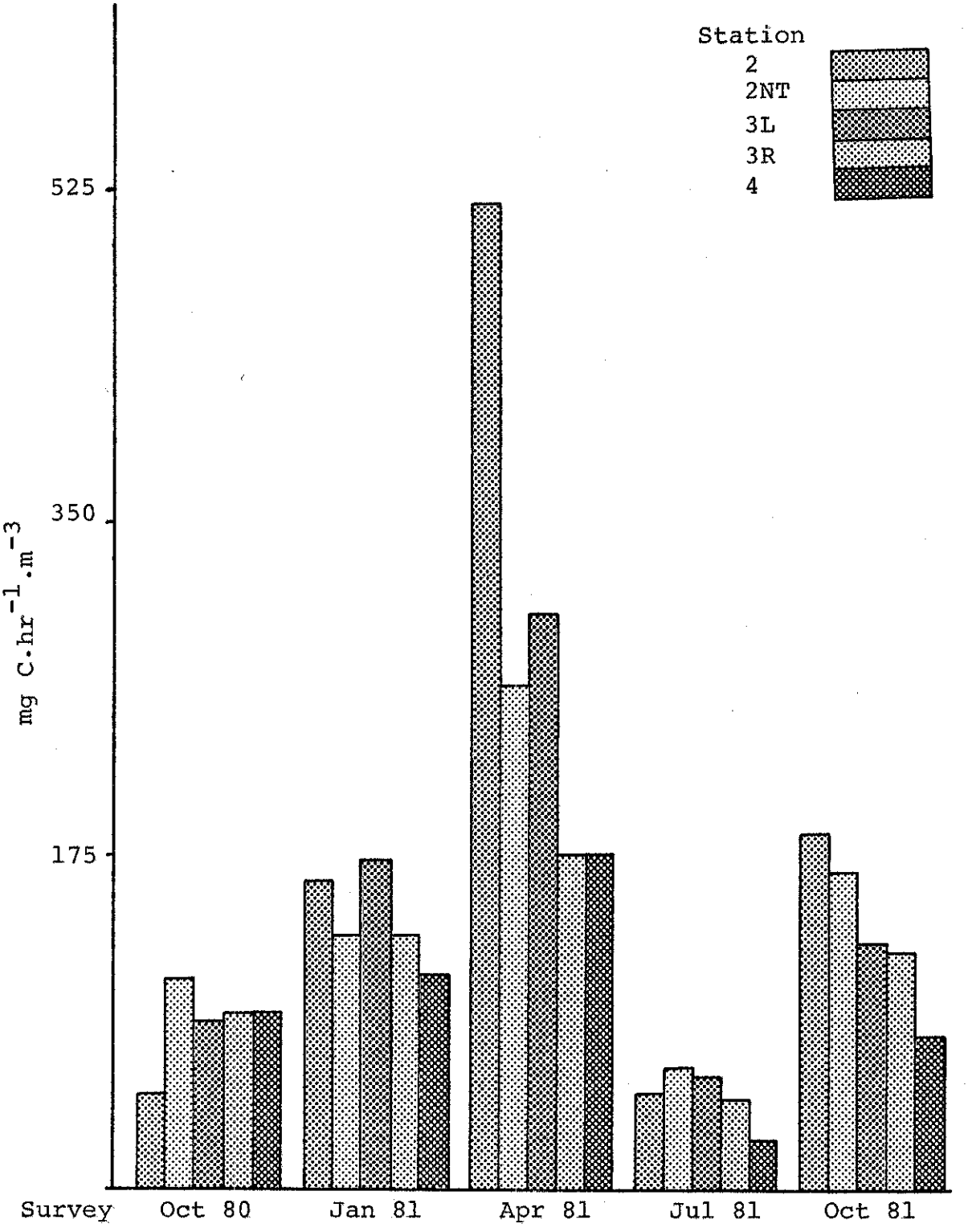


Figure 18. Standard incubation, mean productivity rates (n = 3) for each station and each survey.

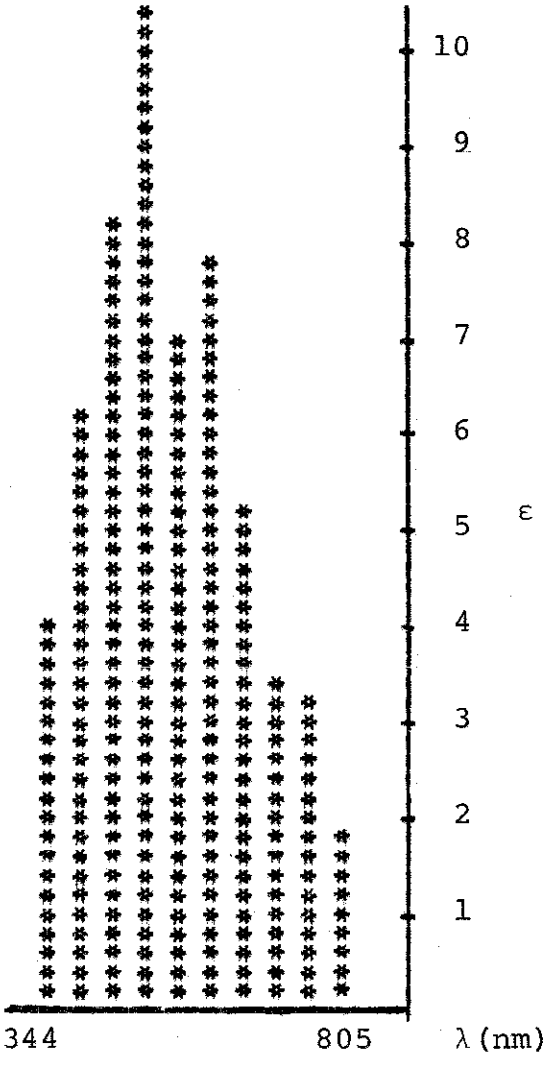


Figure 19. Field measurement of light attenuation by wastewater.

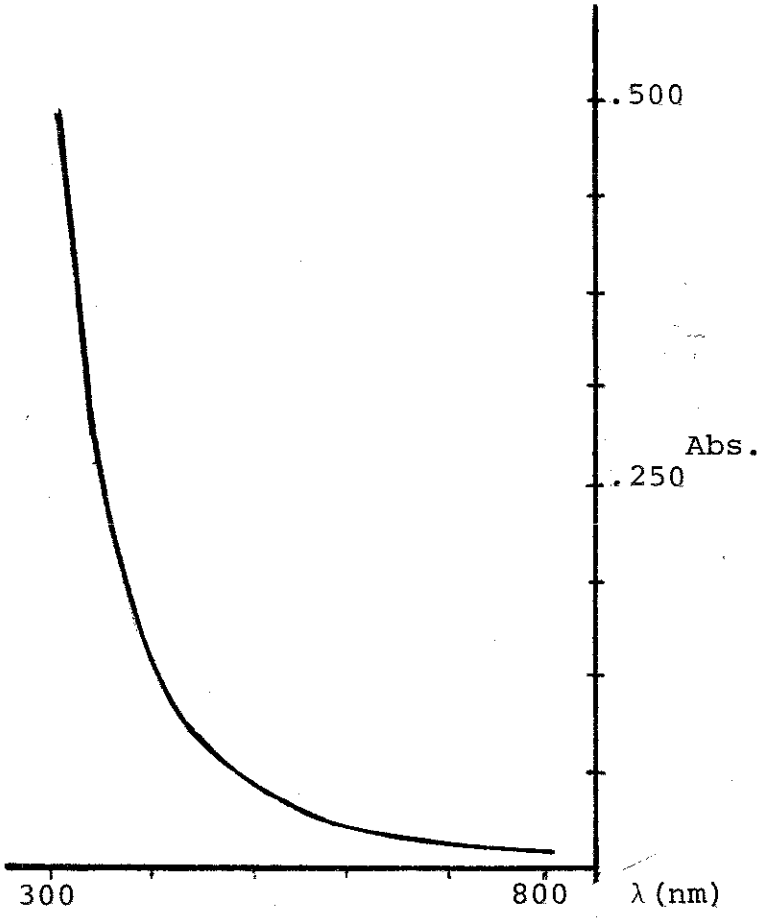


Figure 20. Spectrophotometric absorbance by wastewater diluted with distilled water 1:10 (v/v).

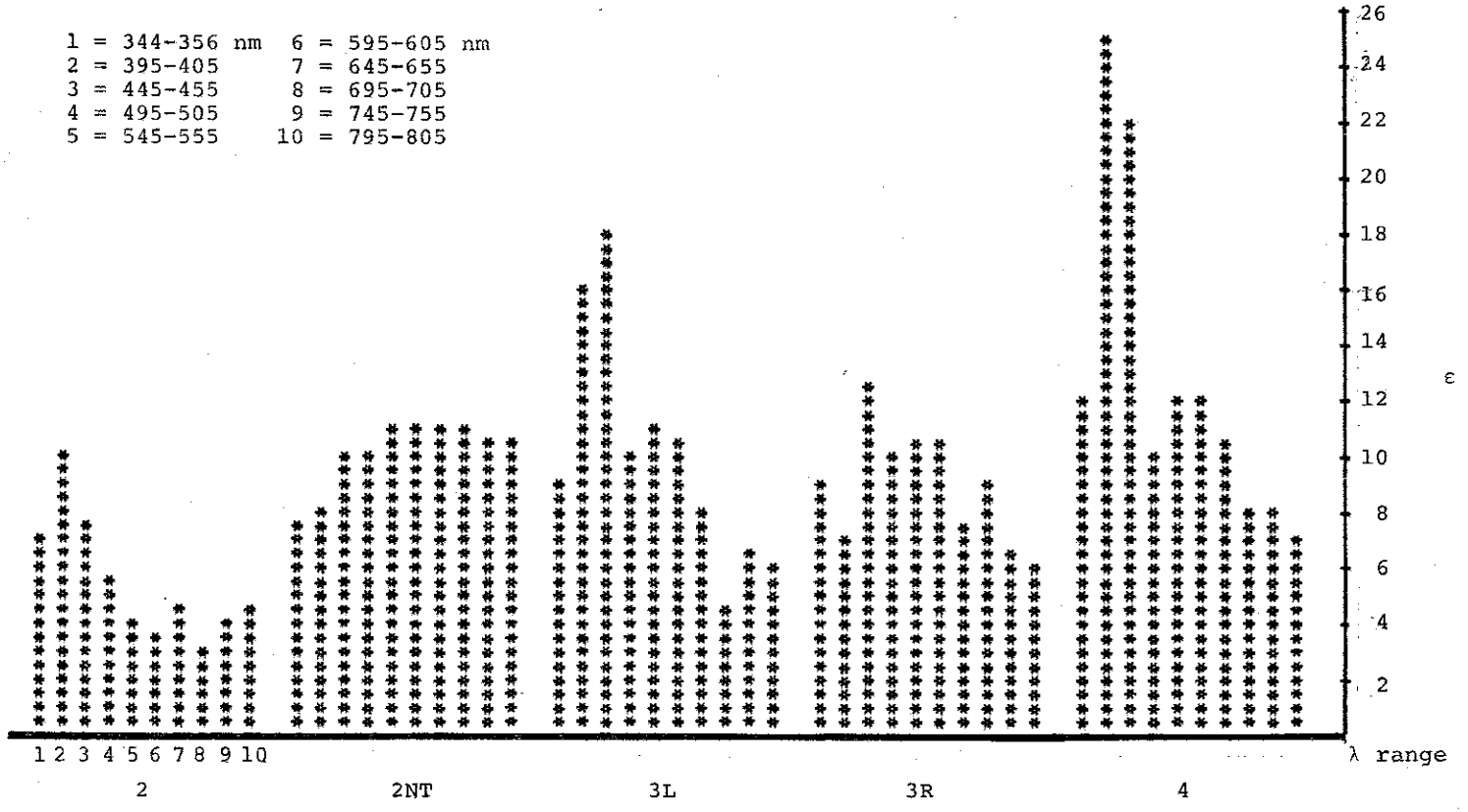


Figure 21. Light quality extinction coefficients at each station for the October 1981 survey.

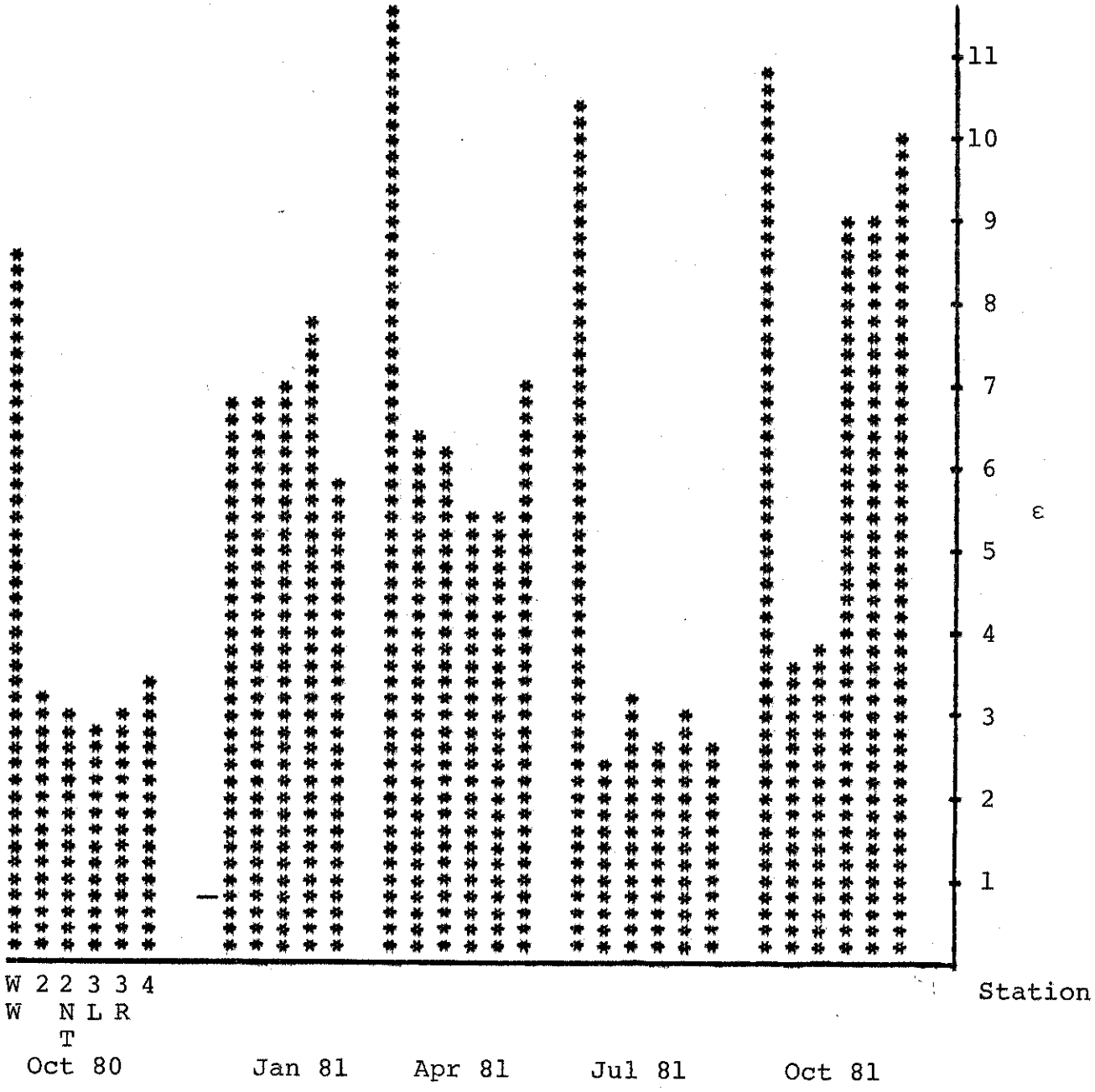


Figure 22. Total PAR extinction coefficients for wastewater and each station during survey months.

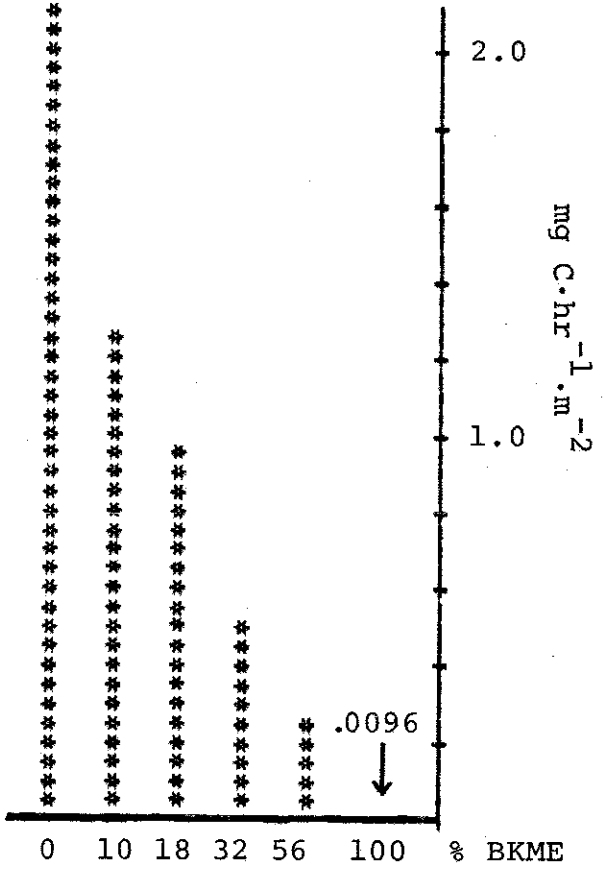


Figure 23. Station 2 periphyton ^{14}C rates measured in each waste dilution. Mean values of 5 runs.

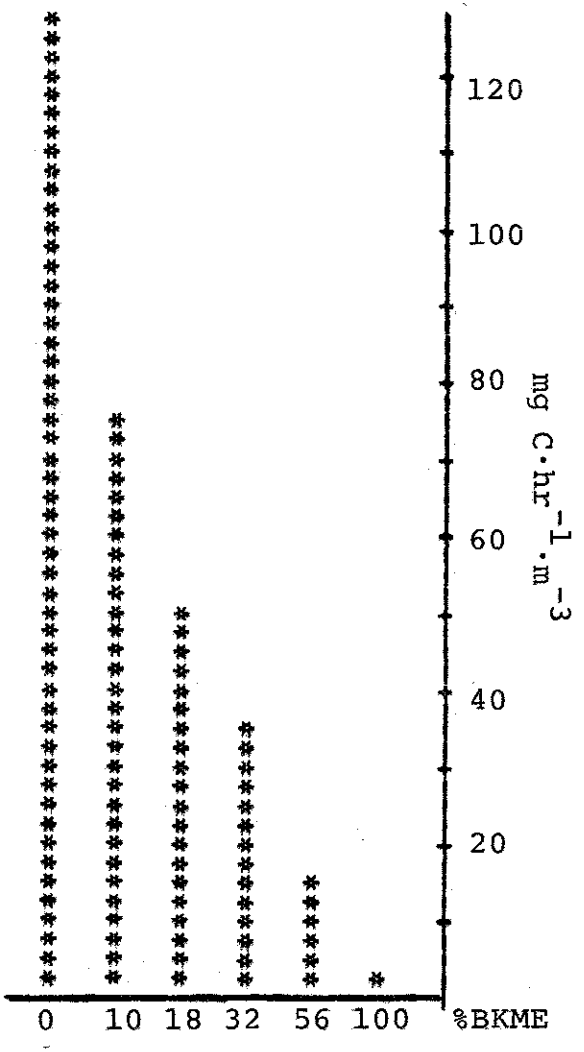


Figure 24. Selenastrum ^{14}C rates measured in each waste dilution. Mean values of 5 runs.

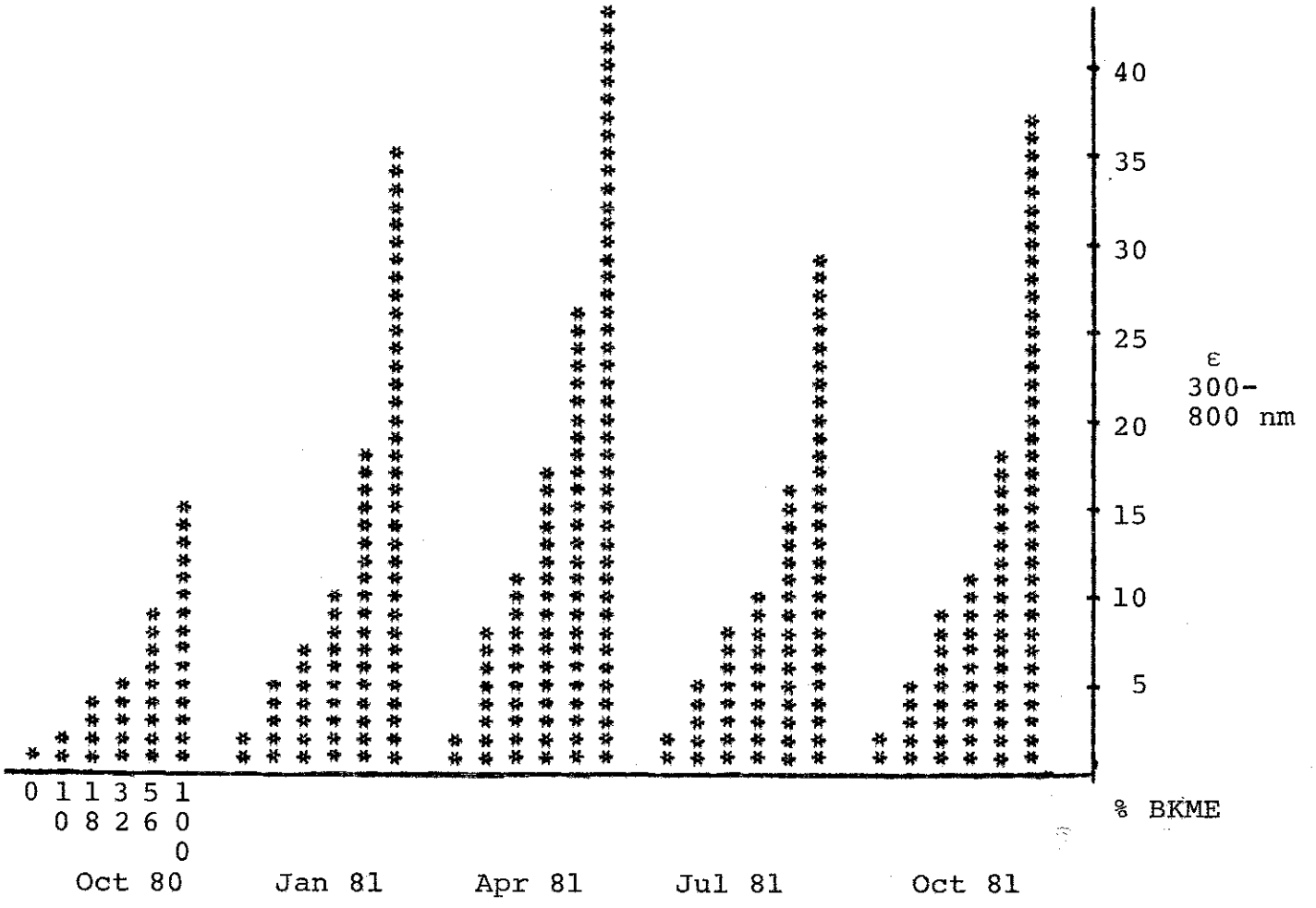


Figure 25. Extinction coefficients, ε, for 300-800 nm wavelength range in color bioassay BKME dilutions. Mean values (n = 3).

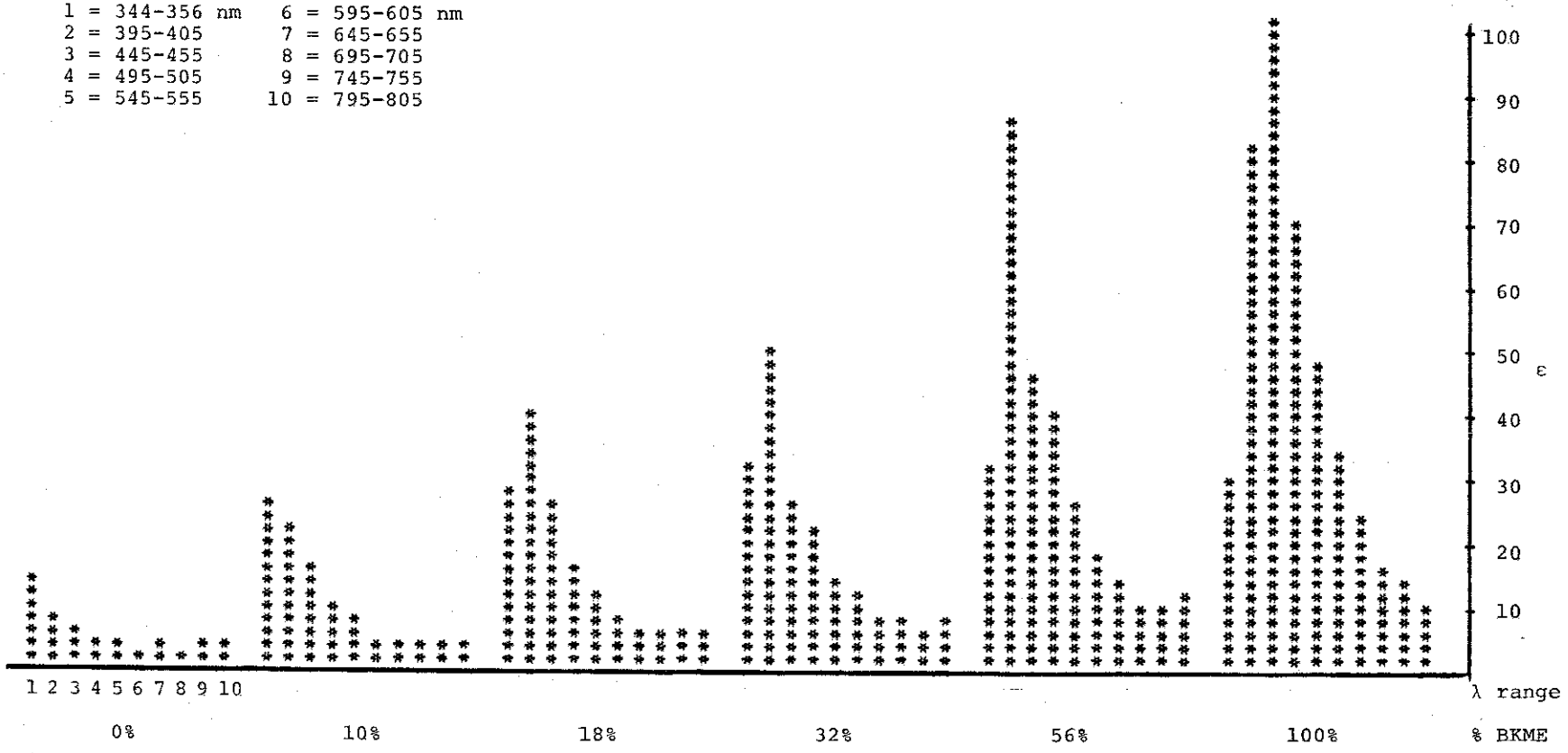


Figure 26. Extinction coefficients, ε, per wavelength range for October 1981 bioassay run. Mean values (n = 3).

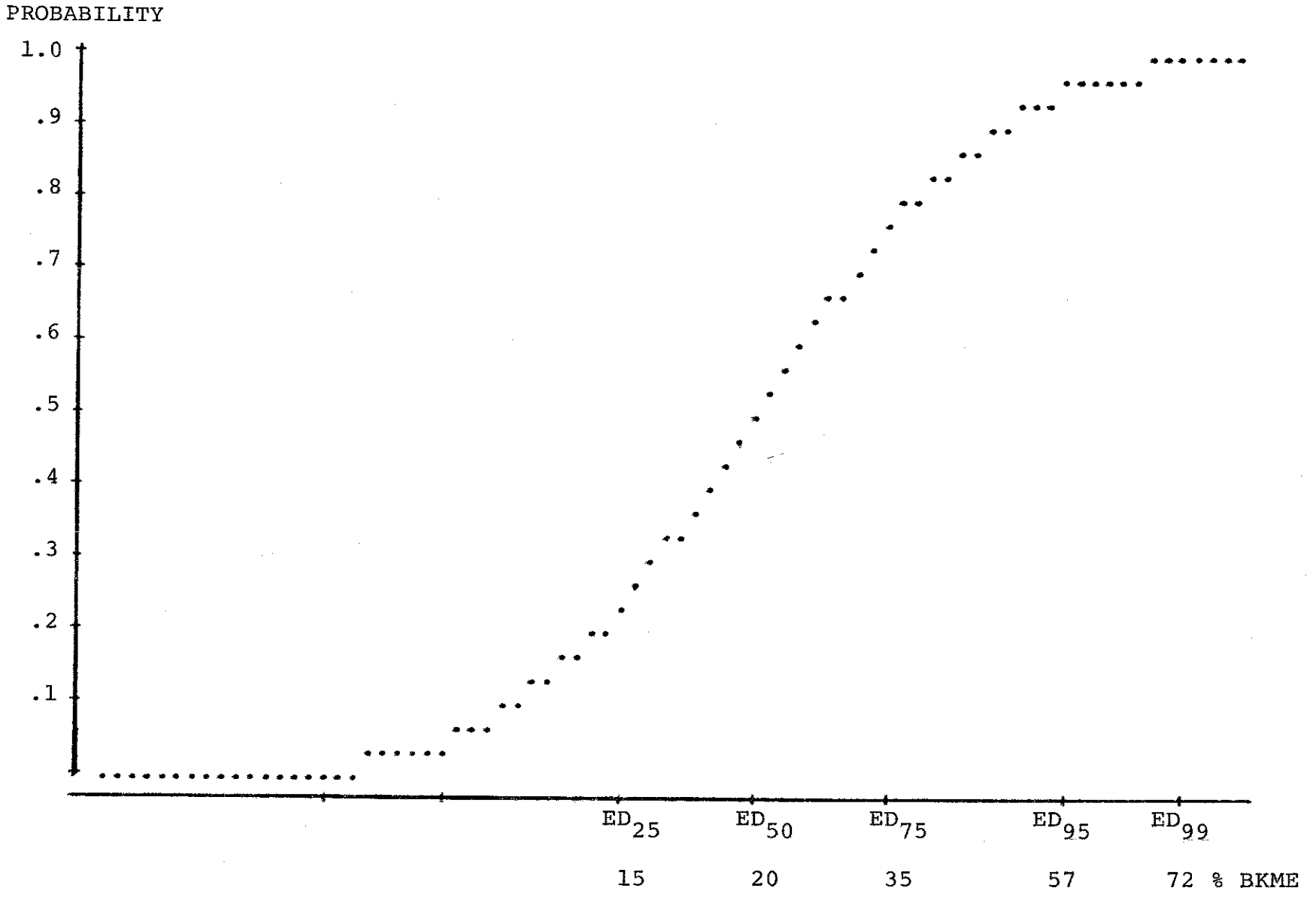


Figure 27. Periphyton rates probability plot. Pooled data of 5 runs

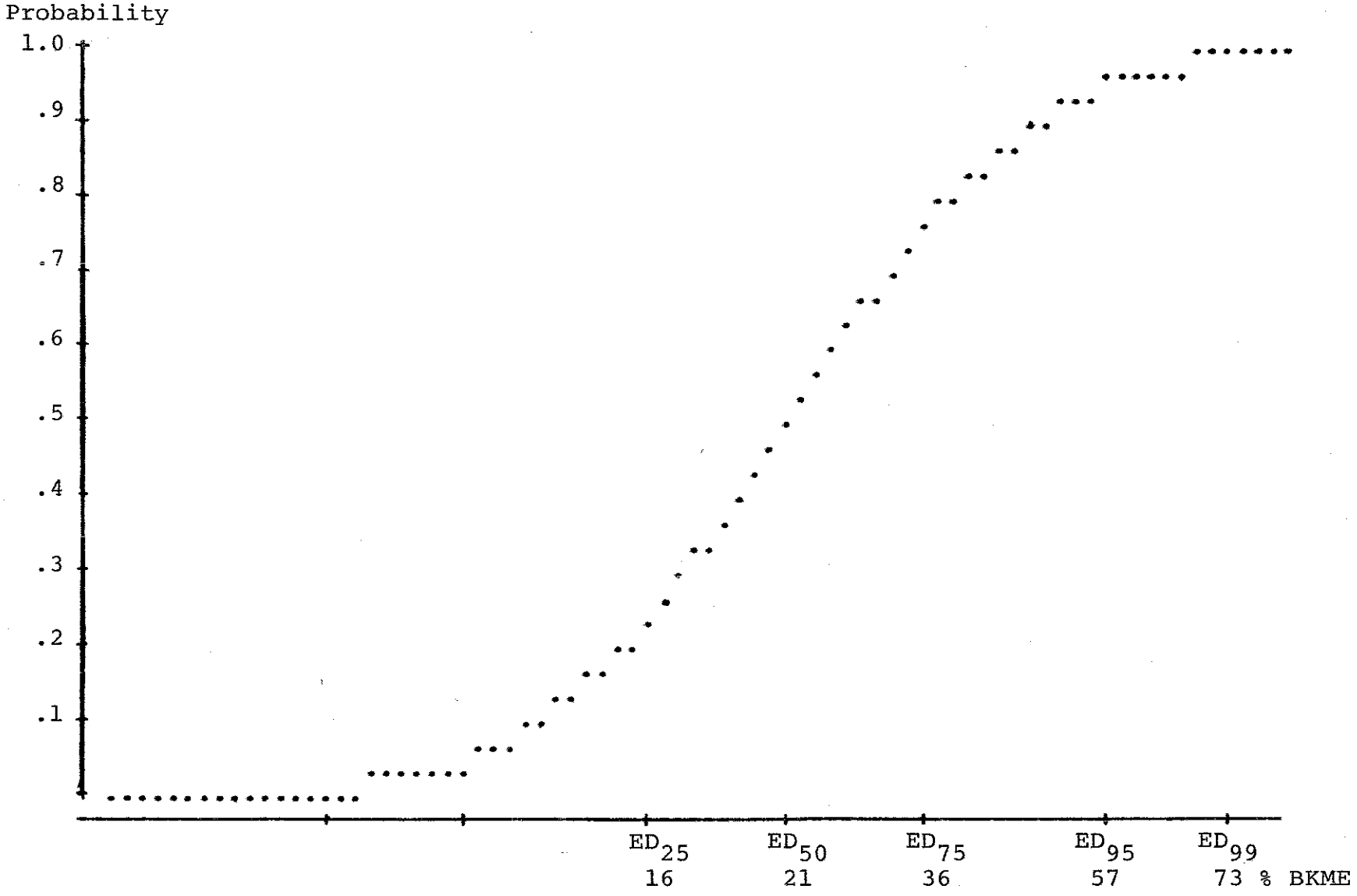


Figure 28. Selenastrum rates probability plot. Pooled data of 5 runs

CHAPTER IV

DISCUSSION

The results of *in situ* experiments indicate that periphyton photosynthetic levels were maintained in the presence of BKME. Obvious differences in light availability and water quality downstream of discharge were found. The consistency of periphyton productivity levels in the face of a drastically altered physical-chemical environment suggests several possible responses of the community to this particular perturbation:

1. If it is assumed that the methods used were sensitive enough to measure actual variation existing between station, then the results do not indicate that BKME was lethal to the periphytic community in general;
2. The quantity of light energy for chlorophyll *a* absorption was equally available and sufficient to drive photosynthesis at all stations. Whether or not this is true would depend on the species composition of the community and the depth of incubation. All incubations were at 5.0 to 10.0 cm below the surface; however, species composition was not determined. Light energy at the absorbance

maxima for chlorophyll a, 445 nm and 665 nm, did decrease downstream and a decrease in productivity at Station 4 was also noted. However, the lower in situ ^{14}C rate at Station 4 was not shown to be significantly different from upstream stations; and,

3. Chromatic adaptation of periphyton species may have occurred, or species with a better suited pigment structure out-competed others less well-equipped or incapable of adaptation. As previously noted, shifts in species composition were identified in response to BKME by Bothwell and Stockner (1980). Species selection and enhanced growth were attributed to increases in nutrients; however, pigment structure, e.g., chlorophyll a:carotenoid ratio, was not measured. The periphyton in this study were not identified nor were accessory pigment concentrations measured. Therefore, the question of chromatic adaptation within species or by interspecies competition in response to BKME remains unresolved. The only data from this study that provide information in this regard were the significant correlations of periphyton PE to the extinction coefficient of the 545 to 555 nm wavelength range ($r = -.535$, significance = .040).

> Algal accessory pigments that can absorb energy from this range are phycobilin (blue-greens) and fucoxanthin (diatoms) (Golterman, 1975).

Periphyton structural index values were highly variable during discharge and non-discharge surveys alike. High variability in periphyton structure when compared to function was also found in artificial stream studies of Rodgers et al. (1979). Their results did not, however, show this variability to be associated with perturbations. In this present study, lower structural index values (increased heterotrophic component) were highly correlated to increased DOC concentration ($r = -.740$, significance = .003). The lowest structural index value and highest DOC concentrations during discharge were found at Station 3R, the most proximate to the mill outfall.

Contrary to the periphyton results, phytoplankton photosynthetic rates were significantly lower downstream relative to upstream sites. Relatively equal chlorophyll a concentrations were found for all stations. These data, therefore, suggest that the decrease in productivity was not the result of decreased biomass; i.e., BKME lethality. Significant inverse relationships found with increased light attenuation indicators (ε and selected water chemistry) may indicate that variations in light availability are

associated with changes in phytoplankton ^{14}C rates. This result agrees with previous findings of BKME influence on phytoplankton productivity (Parker and Sibert, 1975; Mechenich, unpublished, 1980). Apparently, the phytoplankton community, originating from upstream sources, was not capable of adapting to the altered downstream light regime as, perhaps, periphyton communities can. On the other hand, free-floating populations sampled at Station 4 may not have had sufficient time to recover (retention time), either by species succession or adaptation (Hynes, 1970).

Laboratory Experiments

The results of the color bioassay experiments adequately demonstrated the effect of light attenuation on photosynthesis. Problems or shortcomings in the predictive ability of this method can not be entirely identified from the results of only one, in situ discharge comparison. However, the apparent potential of periphytic communities to successfully adapt to a BKME perturbation suggests some other applications of the same experimental design, as well as some other supporting studies:

1. ^{14}C productivity of replicate, downstream periphyton communities exposed to BKME can be determined and compared to the upstream replicate

rates. Successful downstream chromatic adaptation may then be indicated by higher rates;

2. Assay of upstream and downstream periphyton rates from replicates incubated within wastewater dilutions may indicate possible BKME impacts on photosynthesis other than light attenuation and possibly account for deviations in model predictions;
3. Pigment composition, particularly chlorophyll *a*: carotenoid ratios, should be determined in future bioassays and *in situ* studies (Welschmeyer and Lorenzen, 1981); and
4. Identification of periphyton species and other qualitative assessments may indicate whether BKME exerts selective pressures between and/or within species populations.

CHAPTER V

CONCLUSIONS

On the basis of the October 1981 survey results, the following conclusions are presented:

1. Primary productivity of periphyton in the lower Sulphur River (mg $C \cdot m^{-2} \cdot hr^{-1}$) was not significantly altered by the presence of the IP Texarkana Mill's wastewater;
2. Periphyton productivity efficiencies (mg $C \cdot hr^{-1}$/ mg chl*a*) were not significantly altered by BKME downstream. This was a reuslt of consistent productivity rates and chlorophyll *a* concentrations between stations;
3. Periphyton community structure shifts significantly toward heterotrophic populations in the immediate vicinity of the mill outfall. Community structural index recovers to upstream levels at Station 4;
4. Phytoplankton primary productivity (mg $C \cdot m^{-3} \cdot hr^{-1}$) was significantly reduced downstream of the mill discharge relative to upstream sites. This decrease is apparently associated with increased light attenuation downstream;

5. The phytoplankton PE ratio (mg $C \cdot hr^{-1}$/mg chl*a*) was reduced downstream of the mill discharge as a result of significant decreases in phytoplankton productivity rates;
6. Station 2 periphyton and *Selenastrum capricornutum* Prinz. ^{14}C-assimilation rates were significantly reduced by BKME-dependent light attenuation;
7. The incubation of indigenous phytoplankton in standard laboratory conditions may have application for seasonal comparisons of primary productivity;
8. In general, decreases in light quantity and quality were correlated with decreases in primary productivity by *in situ* phytoplankton and color bioassay test organisms. Water quality parameters that indicated the light absorption capacity of *in situ* and bioassay water columns (i.e., solids, color, organics, but not necessarily turbidity) were negatively correlated with *in situ* phytoplankton and bioassay productivity rates. Variations in *in situ* periphyton productivity were not found to be correlated with physical-chemical parameters; and
9. The ability of the laboratory color bioassay procedure to predict *in situ* primary productivity responses to BKME was not statistically determined as a result of a lack of *in situ* observations.

However, the generally close agreement between predicted and observed productivity reductions indicate the potential use of the bioassay as an impact management tool. In addition, this design may be useful in elucidating possible community mechanisms of adaptation to BKME perturbations.

Appendix A. Physical-chemical data. Field measurements - one measurement per station. Other parameters - three replicates per station.

Survey	Station	Temp. (°C)	pH	DO (mg/ℓ)	Conduc-tivity (µS)	Cur. Vel. mm/min)	Surface Down-Welling (W/m^2)	Surface Up- (W/m^2)	1 Meter Down-Welling (W/m^2)	1 Meter Up- (W/m^2)	Total PAR ε
Oct 80	WW	23.0	6.96	1.0	3400	.	3.96	0.0008	0.0060	0.0016	8.5071
	2	18.0	8.12	7.8	195	18.7	156.00	6.0000	9.6000	0.8000	3.2581
	2NT	18.2	7.87	7.8	202	19.6	188.00	9.2000	14.4000	1.0000	3.0172
	3L	18.0	8.18	7.8	200	10.8	128.00	7.6000	14.0000	0.9200	2.8239
	3R	18.0	7.83	7.4	220	7.8	7.20	0.3680	0.7200	0.0560	2.9738
	4	19.0	7.80	7.6	210	18.5	116.00	4.0000	11.6000	0.5200	3.3673
Jan 81	WW	7.0	7.32	4.0	2300	.	26.40	0.0000	0.0016	0.0000	.
	2	6.0	7.14	10.9	145	5.4	136.00	0.1520	3.8000	0.0300	6.7965
	2NT	5.5	7.16	10.8	160	0.0	136.00	0.1520	3.8000	0.0300	6.7965
	3L	6.0	7.08	11.0	210	1.0	112.00	0.1080	8.8000	0.0108	6.9441
	3R	6.0	7.03	10.6	210	2.3	116.00	0.0480	7.6000	0.0060	7.7901
	4	7.0	7.28	9.4	170	1.2	44.00	0.1280	4.4000	0.0052	5.8399
Apr 81	WW	16.0	7.40	3.2	2600	.	80.00	0.0008	0.0280	0.0028	11.5129
	2	18.0	7.42	8.4	200	1.5	164.00	0.2960	19.6000	0.0640	6.3173
	2NT	18.0	6.38	8.1	205	1.3	164.00	0.3040	15.6000	0.0560	6.2906

Appendix A--Continued

Survey	Station	Temp	pH	DO	Cond	Speed	LSI	LSR	L1I	L1R	Total PAR ε
Apr 81 cont.	3L	18.0	7.40	9.2	210	.	160.00	0.7200	22.0000	0.1000	5.4037
	3R	18.0	7.35	9.6	220	.	140.00	0.6000	19.2000	0.1120	5.4525
	4	18.0	7.25	7.2	200	.	152.00	0.1280	9.6000	0.0256	7.0796
Jul 81	WW	28.0	6.50	2.8	3200	.	76.00	0.0024	0.5360	0.0016	10.3630
	2	27.0	6.20	3.8	185	30.4	80.00	7.6000	1.4000	0.3160	2.3539
	2NT	27.0	6.10	3.6	185	31.9	44.00	1.7200	0.9600	0.1880	3.2419
	3L	27.0	6.20	3.6	180	41.3	16.80	1.2800	0.5600	0.1080	2.5745
	3R	27.0	6.20	3.6	185	38.4	17.60	0.8800	0.6000	0.1080	2.9957
	4	27.0	6.60	2.9	190	12.3	32.40	2.5200	1.1200	0.1880	2.5539
Oct 81	WW	25.0	7.15	1.6	3100	.	200.00	0.0040	0.0720	0.0028	10.8198
	2	23.0	6.95	4.6	200	14.2	80.00	2.3200	8.8000	0.3000	3.5405
	2NT	24.0	6.96	4.6	200	9.0	72.00	1.6800	7.2000	0.2120	3.7579
	3L	24.0	7.06	4.7	700	2.3	92.00	0.0104	2.2000	0.0020	9.0877
	3R	23.0	7.07	4.7	700	6.3	100.00	0.0124	2.0400	0.0020	8.9952
	4	24.0	6.92	4.6	250	12.7	92.00	0.0044	1.7600	0.0020	9.9479

Appendix A--Continued

Survey	Station	Rep	Acidity (mg/ℓ)	Alkalinity (mg/ℓ)	Hardness (mg/ℓ)	Turbidity (NTV)	TDS (mg/ℓ)	TSS (mg/ℓ)	TOC (mg/ℓ)	DOC (mg/ℓ)	BOD (mg/ℓ)
Oct 80	WW	A	3	240	420	11.0	2310	32	40	34	2.5
	WW	B	5	245	436	11.0	2316	12	40	34	2.0
	WW	C	5	230	420	11.0	2276	20	33	27	1.8
	2	A	4	75	80	31.0	316	38	10	8	3.5
	2	B	3	75	80	31.0	264	10	5	3	2.3
	2	C	4	70	80	28.0	262	24	8	5	2.2
	2NT	A	3	65	84	30.0	288	14	7	6	2.1
	2NT	B	3	70	88	30.0	300	18	7	5	3.8
	2NT	C	4	75	88	30.0	320	40	6	3	2.5
	3L	A	2	70	92	37.0	356	38	4	2	2.0
	3L	B	3	70	100	37.0	336	32	6	2	2.2
	3L	C	3	80	104	37.0	372	34	7	5	2.2
	3R	A	3	70	104	34.0	352	20	5	3	2.5
	3R	B	3	75	100	34.0	388	32	6	5	2.4
	3R	C	3	75	100	34.0	322	30	6	6	2.1
	4	A	3	70	84	31.0	228	26	11	9	2.3
	4	B	2	65	84	32.0	286	26	10	8	2.1
	4	C	2	70	88	32.0	266	18	10	8	1.6
Jan 81	WW	A	46	260	504	7.0	2358	16	60	42	15
	WW	B	44	250	504	6.0	2350	12	65	40	18
	WW	C	39	250	520	7.0	2358	12	70	38	17
	2	A	6	50	84	7.0	144	44	9	8	5.0
	2	B	5	80	156	4.0	156	32	7	7	5.0
	2	C	5	80	92	4.0	152	34	6	6	4.4
	2NT	A	5	75	72	8.0	146	28	7	5	4.3
	2NT	B	4	70	76	9.0	168	28	6	5	4.7
	2NT	C	5	75	76	8.0	158	28	5	2	4.8

Appendix A--Continued

Survey	Station	Rep	Acidity	Alka-linity	Hard-ness	Tur-bidity	TDS	TSS	TOC	DOC	BOD
Jan 81	3L	A	4	70	76	3.0	158	28	8	8	4.5
cont.	3L	B	5	75	80	4.0	150	20	10	7	4.6
	3L	C	5	75	80	3.0	166	26	11	6	4.1
	3R	A	5	75	76	4.0	154	26	5	2	4.7
	3R	B	5	75	72	6.0	148	18	6	4	4.5
	3R	C	5	90	76	4.0	146	26	6	3	4.6
	4	A	5	70	79	8.0	146	32	4	2	4.5
	4	B	6	75	80	8.0	154	28	7	2	4.4
	4	C	6	75	80	9.0	159	23	6	2	4.2
Apr 81	WW	A	10	230	488	13.0	122	14	90	36	4.0
	WW	B	14	235	452	10.0	134	6	88	36	2.8
	WW	C	11	235	452	10.0	138	4	100	35	3.2
	2	A	7	65	84	28.0	168	68	7	6	5.2
	2	B	7	65	84	28.0	170	58	8	6	4.6
	2	C	8	60	88	26.0	162	66	7	6	4.9
	2NT	A	5	60	84	30.0	168	68	9	7	4.3
	2NT	B	6	60	80	31.0	190	68	9	7	4.4
	2NT	C	6	65	84	30.0	176	64	9	7	4.2
	3L	A	6	65	84	30.0	156	60	13	5	5.6
	3L	B	7	65	84	30.0	180	54	13	5	5.7
	3L	C	6	60	80	26.0	172	44	14	5	6.6
	3R	A	7	60	88	29.0	82	50	11	4	6.3
	3R	B	6	60	88	28.0	84	50	11	4	5.7
	3R	C	6	65	88	29.0	66	52	10	5	5.7
	4	A	6	60	84	38.0	58	72	13	11	4.8
	4	B	6	55	84	39.0	74	84	14	10	4.5
	4	C	5	55	72	39.0	34	74	13	9	3.9

Appendix A--Continued.

Survey	Station	Rep	Acidity	Alka-linity	Hard-ness	Tur-bidity	TDS	TSS	TOC	DOC	BOD
Jul 81	WW	A	11	180	660	2.0	1568	20	92	70	0.0
	WW	B	11	175	640	2.0	1920	10	92	70	.
	WW	C	11	180	640	2.0	1900	10	92	70	.
	2	A	8	70	76	3.0	146	6	12	11	0.0
	2	B	8	70	76	3.0	158	8	12	11	.
	2	C	9	70	72	4.0	156	8	12	11	.
	2NT	A	9	70	74	4.0	164	8	12	12	0.0
	2NT	B	8	75	76	4.0	162	8	12	12	.
	2NT	C	6	70	74	4.0	156	6	13	12	.
	3L	A	8	70	76	4.0	168	4	11	10	0.0
	3L	B	7	75	76	4.0	172	4	11	10	.
	3L	C	8	70	76	4.0	172	6	12	10	.
	3R	A	7	70	76	4.0	158	10	10	10	0.0
	3R	B	7	75	76	4.0	180	8	10	10	.
	3R	C	6	70	76	4.0	162	8	10	10	.
	4	A	6	70	76	4.0	178	8	10	10	0.0
	4	B	6	70	76	4.0	178	6	10	10	.
	4	C	7	75	76	4.0	166	10	10	10	.
Oct 81	WW	A	12	180	536	2.9	1757	17	110	100	10
	WW	B	13	180	456	2.9	.	.	.	.	11
	WW	C	12	180	448	3.0	1759	17	.	.	9.3
	2	A	6	80	72	18.0	173	29	9	6	2.9
	2	B	6	75	72	19.0	134	31	9	6	2.6
	2	C	6	75	80	20.0	136	33	9	6	3.3
	2NT	A	4	75	76	20.0	168	36	7	6	2.6
	2NT	B	5	75	76	19.0	131	34	7	6	3.6
	2NT	C	3	75	80	20.0	153	38	8	5	3.3
	3L	A	6	90	132	17.0	370	22	16	12	3.8
	3L	B	9	90	136	17.0	383	28	16	13	3.9
	3L	C	6	90	132	17.0	361	30	16	14	4.5
	3R	A	6	90	128	17.0	367	64	19	16	4.7
	3R	B	6	85	148	17.0	370	18	19	16	4.4
	3R	C	6	95	124	17.0	.	31	19	16	4.5
	4	A	5	75	92	35.0	191	65	10	5	3.6
	4	B	4	75	80	35.0	164	79	10	5	3.3
	4	C	6	75	84	37.0	199	79	10	5	3.2

Appendix A--Continued.

Survey	Station	Rep	Chloride (Cl) (mg/ℓ)	Sulfates (SO_4) (mg/ℓ)	Ammonia (NH_4) (mg/ℓ)	Nitrate (NO_3) (mg/ℓ)	Ortho-Phosphates ($O\text{-}PO_4$) (µg/ℓ)	Total Phosphates ($T\text{-}PO_4$) (mg/ℓ)	Apparent Color (C.V.)	True Color (C.V.)
Oct 80	WW	A	1200	2000	5.9	1.0	0.287	0.567	1300	1200
	WW	B	1240	2000	4.3	1.0	0.275	0.570	1100	1000
	WW	C	1190	2000	4.1	1.0	0.285	0.576	1200	1000
	2	A	21	28	0.0	0.1	0.030	0.101	45	20
	2	B	26	24	0.0	0.1	0.032	0.077	40	20
	2	C	26	23	0.0	0.1	0.039	0.088	40	20
	2NT	A	25	25	0.0	0.1	0.035	0.088	35	20
	2NT	B	25	24	0.0	0.1	0.036	0.088	35	20
	2NT	C	24	24	0.0	0.1	0.046	0.082	35	20
	3L	A	24	27	0.0	0.1	0.032	0.079	40	20
	3L	B	27	25	0.0	0.1	0.027	0.079	40	20
	3L	C	26	27	0.0	0.1	0.038	0.079	40	20
	3R	A	25	27	0.0	0.1	0.035	0.082	40	20
	3R	B	26	26	0.0	0.1	0.032	0.080	40	20
	3R	C	25	28	0.0	0.1	0.033	0.085	40	20
	4	A	24	24	0.0	0.1	0.049	0.086	45	20
	4	B	26	31	0.0	0.1	0.038	0.090	45	20
	4	C	26	30	0.0	0.1	0.033	0.085	45	20
Jan 81	WW	A	960	377	0.8	0.4	0.509	1.230	1375	1000
	WW	B	863	310	0.4	0.3	0.520	1.130	1375	1000
	WW	C	980	310	0.4	0.3	0.463	1.340	1375	1000
	2	A	17	31	0.0	0.1	0.071	0.065	60	40
	2	B	20	28	0.0	0.1	0.380	0.694	70	40
	2	C	22	26	0.0	0.1	0.076	0.055	70	40
	2NT	A	34	40	0.0	0.1	0.084	0.065	60	40
	2NT	B	39	26	0.0	0.1	0.073	0.081	60	40
	2NT	C	44	43	0.0	0.1	0.070	0.078	50	40
	3L	A	101	67	0.0	0.1	0.082	0.143	70	60
	3L	B	118	50	0.0	0.1	0.095	0.136	80	60
	3L	C	132	49	0.0	0.1	0.101	0.136	70	60

Appendix A--Continued.

Survey	Station	Rep	Cl	SO_4	NH_4	NO_3	$O\text{-}PO_4$	$T\text{-}PO_4$	Apparent Color	True Color
Jan 81 cont.	3R	A	159	49	0.0	0.1	0.092	0.120	100	80
	3R	B	185	50	0.0	0.1	0.084	0.107	100	80
	3R	C	218	57	0.0	0.1	0.088	0.120	100	80
	4	A	208	44	0.0	0.1	0.078	0.114	100	80
	4	B	238	33	0.0	0.1	0.114	0.194	100	60
	4	C	270	42	0.0	0.1	0.081	0.104	90	60
Apr 81	WW	A	980	422	1.2	3.4	0.478	0.700	1750	1500
	WW	B	940	565	1.1	3.9	0.490	0.680	1750	1500
	WW	C	920	574	1.2	4.1	0.490	0.704	1750	1500
	2	A	26	51	0.0	0.2	0.268	0.327	80	40
	2	B	30	46	0.0	0.2	0.073	0.158	80	40
	2	C	35	31	0.0	0.1	0.084	0.126	80	40
	2NT	A	35	51	0.0	0.2	0.109	0.170	80	40
	2NT	B	36	46	0.0	0.2	0.088	0.157	80	40
	2NT	C	37	44	0.0	0.2	0.099	0.120	80	40
	3L	A	38	48	0.0	0.2	0.090	0.177	80	40
	3L	B	39	50	0.0	0.1	0.091	0.155	80	40
	3L	C	39	57	0.0	0.1	0.078	0.161	80	40
	3R	A	38	52	0.0	0.1	0.090	0.140	80	40
	3R	B	39	54	0.0	0.1	0.073	0.142	80	80
	3R	C	37	50	0.0	0.1	0.109	0.142	80	80
	4	A	38	58	0.0	0.1	0.156	0.199	120	80
	4	B	37	58	0.0	0.2	0.115	0.194	120	80
	4	C	38	55	0.0	0.1	0.103	0.171	120	80
Jul 81	WW	A	725	22	1.3	0.9	0.335	0.475	1250	1125
	WW	B	721	22	1.3	0.8	0.412	0.574	1250	1125
	WW	C	938	22	1.3	0.8	0.339	0.468	1250	1125
	2	A	9	14	0.0	0.2	0.128	0.179	70	60
	2	B	8	14	0.0	0.2	0.131	0.183	70	60
	2	C	8	13	0.0	0.1	0.141	0.190	70	60

Appendix A--Continued.

Survey	Station	Rep	Cl	SO_4	NH_4	NO_3	$O\text{-}PO_4$	$T\text{-}PO_4$	Apparent Color	True Color
Jul 81	2NT	A	8	13	0.0	0.1	0.162	0.207	70	60
cont.	2NT	B	9	14	0.0	0.1	0.154	0.201	70	60
	2NT	C	9	14	0.0	0.1	0.145	0.207	70	60
	3L	A	8	13	0.0	0.1	0.177	0.207	70	60
	3L	B	8	14	0.0	0.1	0.170	0.210	70	60
	3L	C	8	14	0.0	0.1	0.166	0.203	70	60
	3R	A	8	14	0.0	0.1	0.168	0.214	70	60
	3R	B	9	13	0.0	0.1	0.173	0.212	70	60
	3R	C	8	13	0.0	0.1	0.171	0.214	70	60
	4	A	8	14	0.0	0.1	0.162	0.216	70	60
	4	B	8	14	0.0	0.1	0.171	0.219	70	60
	4	C	8	14	0.0	0.1	0.177	0.218	70	60
Oct 81	WW	A	600	304	1.3	1.5	0.312	0.431	1250	1000
	WW	B	590	296	1.3	1.5	0.344	0.501	1250	1000
	WW	C	610	298	1.3	1.5	0.285	0.458	1250	1000
	2	A	13	9	0.0	0.1	0.215	0.243	55	30
	2	B	13	9	0.0	0.1	0.074	0.114	55	30
	2	C	13	9	0.0	0.1	0.051	0.112	55	30
	2NT	A	13	10	0.0	0.1	0.070	0.117	55	30
	2NT	B	13	10	0.0	0.1	0.076	0.102	55	30
	2NT	C	13	10	0.0	0.1	0.084	0.118	55	30
	3L	A	128	60	0.2	0.4	0.126	0.174	225	175
	3L	B	130	57	0.2	0.4	0.121	0.168	225	175
	3L	C	130	57	0.2	0.4	0.119	0.163	225	175
	3R	A	120	55	0.0	0.4	0.133	0.167	225	175
	3R	B	120	52	0.0	0.4	0.102	0.154	225	175
	3R	C	118	52	0.0	0.4	0.107	0.165	225	175
	4	A	25	15	0.0	0.1	0.110	0.180	100	60
	4	B	25	16	0.0	0.2	0.116	0.194	100	60
	4	C	25	16	0.0	0.2	0.105	0.168	100	60

Appendix B. Calcualted primary productivity data. Three replicates per station.

			Periphyton					Phytoplankton			
Survey	Sta.	Rep	(in situ rate)	(PE)	Chl a	AFDRYWT ($x10^4$)	S.I. ($x10^3$)	(in situ rate)	PE ($x10^3$)	Chl a	(std. incub. rate)
Oct 80	2	A	8.372	48.39	0.173	7.42	0.31	54.38	6.80	0.008	93.40
	2	B	5.551	49.56	0.112	3.86	0.20	101.49	11.28	0.009	112.12
	2	C	6.060	15.74	0.385	.	0.68	93.31	11.66	0.008	93.62
	2NT	A	1.177	8.12	0.145	3.42	0.47	197.28	28.18	0.007	104.72
	2NT	B	7.591	75.91	0.100	2.89	0.33	217.05	31.01	0.007	117.44
	2NT	C	10.479	58.54	0.179	2.86	0.58	201.57	28.80	0.007	101.91
	3L	A	4.493	9.70	0.463	7.49	0.76	114.83	14.35	0.008	98.40
	3L	B	3.857	5.95	0.648	5.88	1.07	148.25	21.18	0.007	102.36
	3L	C	3.899	10.43	0.374	4.79	0.62	163.54	23.36	0.007	83.72
	3R	A	1.855	5.27	0.352	8.08	0.73	191.10	19.11	0.010	90.61
	3R	B	1.137	5.66	0.201	3.36	0.42	220.20	27.52	0.008	99.38
	3R	C	0.963	6.17	0.156	3.04	0.32	199.27	22.14	0.009	103.16
	4	A	1.720	12.29	0.140	1.91	0.57	1795.90	299.3	0.006	108.36
	4	B	0.080	0.95	0.084	2.58	0.34	1618.80	202.3	0.008	99.44
	4	C	1.822	9.90	0.184	2.91	0.75	1891.10	236.4	0.008	90.80
Jan 81	2	A	18.296	15.25	1.200	0.89	6.99	116.82	9.73	0.012	179.38
	2	B	3.410	5.14	0.664	1.91	3.87	79.12	11.30	0.007	130.90
	2	C	15.533	32.36	0.480	2.35	2.80	129.46	16.18	0.008	144.75
	2NT	A	1.215	1.15	1.061	2.30	4.94	67.32	6.73	0.010	121.43
	2NT	B	1.300	1.40	0.927	1.88	4.32	69.14	9.88	0.007	138.28
	2NT	C	1.944	1.77	1.100	2.26	5.12	72.31	6.57	0.011	139.63
	3L	A	16.414	20.86	0.787	1.14	8.67	100.95	10.09	0.010	162.37
	3L	B	16.640	24.43	0.681	0.67	7.50	121.96	12.20	0.010	175.81
	3L	C	13.244	19.95	0.664	0.92	7.31	122.91	12.29	0.010	160.15
	3R	A	-1.563	-3.54	0.441	0.98	5.26	124.31	12.43	0.010	180.16
	3R	B	5.921	18.62	0.318	0.67	3.79	117.27	14.66	0.008	109.15
	3R	C	2.370	9.87	0.240	0.87	2.86	125.05	17.86	0.007	106.02
	4	A	8.600	23.69	0.363	1.57	3.61	50.78	6.35	0.008	102.30
	4	B	12.191	14.46	0.843	0.60	8.38	65.42	6.54	0.010	110.71
	4	C	13.130	20.64	0.636	0.85	6.33	84.64	8.46	0.010	118.35

Appendix B--Continued.

			Periphyton					Phytoplankton			
Survey	Sta	Rep	In Situ rate	PE	Chl a	AFDRYWT ($\times 10^4$)	S.I. ($\times 10^3$)	In situ rate	PE ($\times 10^3$)	Chl a	Std. Incub. rate
Apr 81	2	A	1.737	4.44	0.391	362.8	0.02	277.90	12.08	0.023	871.80
	2	B	3.377	9.90	0.341	63.82	0.02	281.28	12.23	0.023	429.85
	2	C	2.316	5.18	0.447	216.8	0.02	244.51	13.58	0.018	233.78
	2NT	A	12.370	24.64	0.502	2.98	1.87	584.18	48.68	0.012	222.38
	2NT	B	6.871	22.83	0.301	2.88	1.12	633.50	52.79	0.012	267.51
	2NT	C	18.727	46.58	0.402	2.19	1.50	664.76	60.43	0.011	292.54
	3L	A	5.341	12.42	0.430	3.30	0.46	305.20	16.96	0.018	135.63
	3L	B	2.821	9.37	0.301	12.67	0.32	307.52	18.09	0.017	595.07
	3L	C	3.456	4.52	0.765	11.94	0.82	285.46	142.7	0.002	161.66
	3R	A	2.647	5.64	0.469	2.93	1.63	291.90	13.90	0.021	149.39
	3R	B	7.279	13.16	0.553	2.38	1.92	165.67	13.81	0.012	208.98
	3R	C	7.705	18.66	0.413	3.34	1.43	306.17	13.92	0.022	159.52
	4	A	3.439	3.92	0.877	15.76	0.48	185.91	10.33	0.018	170.49
	4	B	3.581	6.61	0.542	31.41	0.29	329.39	23.53	0.014	202.69
Jul 81	4	C	4.374	14.25	0.307	7.97	0.17	296.67	21.19	0.014	148.15
	2	A	12.119	77.69	0.156	5.35	0.21	75.25	15.05	0.005	43.05
	2	B	40.164	375.36	0.107	8.63	0.14	95.35	13.62	0.007	57.18
	2	C	31.595	185.85	0.170	8.77	0.22	83.76	13.96	0.006	48.85
	2NT	A	39.562	326.96	0.121	2.74	0.84	121.72	17.39	0.007	64.67
	2NT	B	46.441	305.53	0.152	0.88	1.06	118.95	19.82	0.006	64.38
	2NT	C	116.010	1303.48	0.089	0.68	0.62	163.73	20.47	0.008	54.72
	3L	A	3.216	36.13	0.089	1.63	0.85	105.96	15.14	0.007	33.63
	3L	B	5.585	32.28	0.173	0.63	1.64	105.37	11.71	0.009	51.82
	3L	C	2.697	17.29	0.156	0.90	1.48	111.37	15.91	0.007	81.35
	3R	A	48.737	487.37	0.100	0.92	0.56	100.75	12.59	0.008	43.86
	3R	B	15.606	147.23	0.106	0.91	0.60	102.42	12.80	0.008	42.12
	3R	C	5.367	53.67	0.100	3.51	0.56	108.96	15.57	0.007	42.98
	4	A	1.858	7.74	0.240	2.97	0.78	72.97	24.32	0.003	27.14
	4	B	9.506	26.19	0.363	2.48	1.18	66.27	16.57	0.004	22.02
	4	C	13.861	57.75	0.240	3.80	0.78	73.14	24.38	0.003	22.55

Appendix B--Continued.

			Periphyton					Phytoplankton			
Survey	Sta	Rep	In Situ rate	PE	Chl a	AFDRYWT ($x10^4$)	S.I. ($x10^3$)	In Situ rate	PE ($x10^3$)	Chl a	In Situ rate
Oct 81	2	A	2.655	5.29	0.502	13.57	0.23	420.19	42.02	0.010	180.38
	2	B	2.723	27.23	0.100	10.30	0.05	413.23	29.52	0.014	187.60
	2	C	2.830	16.95	0.167	40.24	0.08	499.15	33.28	0.015	189.05
	2NT	A	4.033	12.68	0.318	5.44	0.55	339.49	18.86	0.018	157.97
	2NT	B	2.329	3.21	0.726	6.81	1.25	514.67	23.39	0.022	161.73
	2NT	C	3.818	19.58	0.195	5.17	0.34	379.43	22.32	0.017	171.76
	3L	A	1.176	4.06	0.290	12.04	0.11	255.75	25.57	0.010	93.26
	3L	B	5.513	13.51	0.408	23.07	0.15	248.15	22.56	0.011	135.44
	3L	C	2.794	8.62	0.324	45.46	0.12	252.45	19.42	0.013	154.86
	3R	A	6.962	27.74	0.251	192.9	0.02	190.13	19.01	0.010	154.91
	3R	B	2.666	13.26	0.201	218.3	0.01	208.34	9.47	0.022	101.97
	3R	C	2.605	6.86	0.380	22.57	0.03	167.52	15.23	0.011	109.59
	4	A	2.388	10.43	0.229	3.91	0.60	156.34	17.37	0.009	78.96
	4	B	0.645	2.69	0.240	4.46	0.63	182.06	16.55	0.011	75.71
	4	C	0.593	2.41	0.246	3.14	0.64	144.34	12.03	0.012	77.42

Appendix C. Analysis of Variance Results for Productivity Parameters During Non-discharge Surveys.

Productivity Parameter	Jul 81 Survey C.V.	Jul 81 Survey Kruskal Wallis	Jul 81 Survey Newman Keuls Groups	Apr 81 Survey C.V.	Apr 81 Survey Kruskal Wallis	Apr 81 Survey Newman Keuls Groups
Periphyton						
^{14}C rate	86	NS	2NT 2 3R 4 3L	53	NS	2NT 3R 4 3L 2
PE	123	.025	2NT 3R 2 4 3L	55	NS	2NT 3R 3L 4 2
Chl a	27	NS	4 2 3L 2NT 3R	38	NS	4 3R 3L 2NT 2
AFDRYWT	42	.05	2 4 3R 2NT 3L	135	.025	2 4 3L 3R 2NT
S.I.	31	.05	3L 4 2NT 3R 2	30	.05	3R 2NT 3L 4 2
Phytoplankton						
^{14}C rate	12	.025	2NT 3L 3R 2 4	15	NS	2NT 3L 4 3R 2
PE	15	.05	4 2NT 3L 2 3R	103	.025	2NT 3L 4 3R 2
Chl a	14	.05	3R 3L 2NT 2 4	32	NS	2 3R 4 3L 2NT
Standard Incub.						
^{14}C rate	25	.05	2NT 3L 2 3R 4	66	NS	2 2NT 3L 3R 4

Appendix C--Continued.

Productivity Parameter	Jan 81 Survey C.V.	Kruskal Wallis	Newman Keuls Groups	Nov 80 Survey C.V.	Kruskal Wallis	Newman Keuls Groups
Periphyton						
^{14}C rate	48	.05	3L 2 4 3R 2NT	58	NS	2 2NT 3L 3R 4
PE	60	NS	3L 2 4 3R 2NT	84	NS	2 2NT 4 3L 3R
Chl a	31	NS	2NT 2 3L 4 3R	42	NS	3L 3R 2 2NT 4
AFDRYWT	33	NS	2NT 2 3L 3R 4	40	NS	3L 3R 2 2NT 4
S.I.	29	NS	3L 4 2NT 2 3R	39	NS	3L 4 3R 2NT 2
Phytoplankton						
^{14}C rate	16	.05	3R 3L 2 2NT 4	13	.025	4 2NT 3R 3L 2
PE	21	.025	3R 2 3L 2NT 4	34	.025	4 2NT 3R 3L 2
Chl a	19	NS	3L 2NT 4 2 3R	10	NS	3R 2 4 3L 2NT
Standard Incub.						
^{14}C rate	16	NS	3L 2 2NT 3R 4	9	NS	2NT 4 2 3R 3L

Appendix D. Fiducial limits of probability plots for Station 2 periphyton and Selenstrum capricorntum Prinz. productivity rates in the color bioassay.

Probability	% BKME	95% Fiducial Limits for Periphyton Lower	Upper	% BKME	95% Fiducial Limits for Selenastrum Lower	Upper
.99	72	48	301	73	48	273
.95	57	37	242	57	38	200
.90	49	32	195	49	33	162
.85	43	28	164	44	29	137
.80	39	25	140	40	28	117
.75	35	22	119	36	22	100
.70	32	18	101	32	19	85
.65	29	15	84	29	15	72
.60	26	10	70	26	11	60
.55	23	5	57	23	6	50
.50	20	-2	47	21	0	43
.45	17	-12	38	16	-11	33
.40	14	-25	31	15	-19	30

Appendix D--Continued.

Probability	%BKME	95% Fiducial Limits for Periphyton Lower	Upper	% BKME	95% Fiducial Limits for Selenastrum Lower	Upper
.35	11	-39	25	12	-30	25
.30	8	-53	22	9	-43	22
.25	5	-70	19	6	-57	19
.20	1	-91	15	2	-74	15
.15	-4	-117	11	-3	-94	12
.10	-9	-147	8	-8	-120	8
.05	-17	-160	3	-16	-158	3
.01	-32	-283	-7	-32	-230	-7

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Phil White, Environmental Officer, International Paper Co., Texarkana Mill, Texarkana, Texas, 1981.