PRIMARY PRODUCTIVITY AND ENERGY RELATIONSHIPS IN ARTIFICIAL STREAMS

Thesis for the Degree of Ph. D.

MICHIGAN STATE UNIVERSITY

Niles R. Kevern

1962

This is to certify that the

thesis entitled

PRIMARY PRODUCTIVITY AND ENERGY
RELATIONSHIPS IN ARTIFICIAL STREAMS

presented by

Niles R. Kevern

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Fisheries & Wildlife

Robert C. Ball
Major professor

Date December 13, 1962

O-169

ABSTRACT

PRIMARY PRODUCTIVITY AND ENERGY RELATIONSHIPS IN ARTIFICIAL STREAMS

by Niles R. Kevern

The productivity and energetics of algal communities in two, recirculating, artificial streams were investigated. Emphases were placed on a comparative study of methods used to measure primary productivity in flowing waters and on the effects of controlled environmental characteristics on energy flow.

The artificial streams, with incandescent lights as the source of energy, were established with distilled water and inorganic nutrients and were seeded with algae from a local stream. Succession ended with a filamentous blue-green alga, Plectonema Boryanum Gomont, as the dominant organism. Productivity of the algae was measured by pH-carbon dioxide changes, upstream-downstream oxygen changes, and by the biomass of periphyton collected on artificial substrates.

Increases in light intensity, current velocity, and chelate concentrations caused significant increases in productivity. An increase in temperature resulted in a Q_{10} of 1.4 for the growth rate of the algae indicating that respiration increased about the same as photosynthesis and causing only a slight increase in net productivity. Alteration of the photoperiod did not cause a significant increase in productivity.

Productivity measured by the biomass of periphyton collected on substrates was generally lower than estimates by the carbon dioxide and oxygen methods, but substrate estimates increased when calculations

were based on the growth rate occurring on the last few days of an extended exposure period. The growth of the periphyton population was described as having a J-shaped form. A close linear relationship was noted between the concentration of phytopigment, extracted by ethanol, and the ash-free dry weight of the periphyton.

Estimates of net productivity by the carbon dioxide and oxygen methods were in general agreement when the oxygen values were corrected by a photosynthetic quotient of 1.1 to 1.3 depending upon the measured caloric value of the algae.

The radiant energy received by the periphyton was about 1% of the average daylight intensity. Radiant energy fixed as the chemical energy of organic matter varied from 0.06 to 0.20 g cal cm^{-2} day^{-1} being somewhat less than the rates of most natural communities. Photosynthetic efficiencies based on gross productivity varied from 2.1 to 12.5%, while those based on net productivity varied from 0.9 to 4.1%. These relatively high efficiencies were explained on the basis of the predominance of red wavelengths in the light received by the periphyton and the low light intensities. High efficiencies generally occurred with high productivities; however, and increase in productivity caused by an increase in light intensity was accompanied by a decrease in efficiency.

The primary source of nutrients during the study periods was from decomposition of periphyton. Phosphorus regeneration was shown to take place readily in the dark of the reservoirs and filters. Phosphorus release from insoluble sources occurred when a chelate, EDTA, was added to the streams and phosphorus was precipitated when suspensions of kaolin were added to the streams under acid conditions.

PRIMARY PRODUCTIVITY AND ENERGY RELATIONSHIPS IN ARTIFICIAL STREAMS

By

Niles R. Kevern

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Fisheries and Wildlife

1962

ACKNOWLEDGMENTS

I offer my sincere thanks to Dr. Robert C. Ball for his supervision of the study, for his guidance as my advisor, and for his unselfish contribution of time.

I wish to thank Drs. Philip J. Clark, Gerald W. Prescott, and Peter I. Tack for their time given as members of my guidance committee. I also thank Dr. Prescott for his aid in taxonomic problems and Dr. Clark for his advice on presentation of statistical analyses.

My appreciation is extended to many of my fellow graduate students for their discussions, opinions, and suggestions relating to the study.

And to my wife, Kay, I give my thanks for her patience and understanding through the period of graduate study.

I am also grateful for financial assistance under Fellowship No. GF-12,649 from the Division of General Medical Sciences of the National Institutes of Health, Department of Health, Education, and Welfare.

****

TABLE OF CONTENTS

TABLE OF CONTENTS - Continued

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES - Continued

FIGURE Page

INTRODUCTION

Primary productivity, the fundamental problem of aquatic biology, is defined by Frey (1956) as radiant energy fixed by photosynthetic plants as chemical energy. This phenomenon has been the subject of extensive study for many years with most attention being centered on lakes and ponds. Primary productivity in flowing waters has received comparable attention only in recent years with many of the methods used to measure production in lakes being found inadequate for stream studies. Development of new methods and techniques for free water was necessary, since the basic difference between lakes and streams is the unidirectional flow of water in streams. These free water methods, that is, methods not requiring isolation of the water mass, are still in a state of development and refinement.

Laboratory experiments employing culture vessels, the Warburg apparatus, and other such tools have contributed significantly to the methodology used in lake studies and to the understanding of phenomena relating to primary productivity in standing waters. Comparable laboratory studies relating to stream productivity, such as the study of a stream microcosm by Odum and Hoskin (1957), have been few. The potential then, remains considerable for the use of artificial streams to investigate the relationships involved in the primary productivity of flowing waters. Many of these relationships of productivity are based on the effects of environmental factors on the metabolism of the producers. It is possible, in a laboratory situation, to isolate or control many of the environmental factors in order to determine their influence on the fixation of energy by autotrophic organisms.

This study of primary productivity in artificial streams was established with three main objectives, 1) to determine the effects of certain environmental factors on productivity, 2) to critically compare and investigate the methods of measuring primary productivity in flowing waters, and 3) to characterize the artificial stream as a research tool for adding to our knowledge of stream ecology.

Environmental factors were controlled and investigated individually with the influence on productivity being measured comparatively by the several methods also under investigation. It was thus possible to evaluate both the environmental effects and the methods. Since productivity studies are best quantified in energy terms, the results of the environmental studies were converted to energy flow and efficiencies and these, in addition to nutrient observations, used to characterize the artificial streams in comparisons with other aquatic ecosystems.

LITERATURE REVIEW

This review is intended to cover the studies in limnology that have dealt with primary production in general and with stream studies in particular.

Early work, from the middle nineteenth century into the first of the twentieth century, was predominantly European and dealt for the most part with plankton studies in lakes. Welch (1951) gives a historical review of limnology and mentions such early European investigators as J. Müller, P. E. Müller, A. Fritsch, F. A. Forel, and F. Simony. Early work in the United States was likewise dominated by studies concerned with plankton in lakes, largely zooplankton as evidenced in the five year review of Ward (1899) and the work of Marsh (1899). Wolle in 1887 published the first notable key to the algae of the United States, but made little mention of limnological relationships. Early United States literature was not without some indication of the studies of production and aquatic ecology which were to follow. S. A. Forbes (1887) gave limnology in the United States much of its ecological bearing and Marsh (1899) mentioned measurement of productiveness as a future need.

During the first part of the 1900's, production studies were numerous enough to prompt several philosophical papers attempting to define, justify, and clarify production and the measurement of production. Transeau (1926) expressed concern over the exhaustion of our stored energy supply and made one of the first estimations of energy fixation by plants. Klugh in 1926 stated the ultimate aim of limnology to be fish production and attempted to infer a productivity index for lakes based upon the rooted aquatic vegetation. Strøm in 1928 spoke of

"production biology" as the biological build-up of organic matter by autotrophic plants, primarily algae, with the final practical aim being fish production. Strøm (op. cit.) based his discussion on the work of many early and well-known investigators such as Naumann, Thienemann, Wesenberg-Lund, Alm, Alsterberg, Utermöhl, Birge and Juday, Atkins, and Harvey. Thienemann in 1931 tried to reconcile the many interpretations of production already existing by that time. In the United States, Viosca (1935) published an essay on the dependence of fish production upon productivity at lower trophic levels.

pH, Carbon Dioxide, and Alkalinity

The general relationship of carbon dioxide and oxygen with photosynthesis has been known since the observations of J. Priestly in the eighteenth century, but it was not until the twentieth century that this relationship was applied significantly in the measurement of primary productivity.

In 1914, Birge and Juday discussed factors involved in producing a stratum of strongly alkaline water. This happened in lakes when a thermocline occurred near the surface thus concentrating photosynthetic organisms which, under favorable conditions, caused a drop in free and half-bound carbon dioxide. No attempt was made to calculate production by this rise of alkalinity. Moore and his colleagues in 1915 noted a marked rise in the pH and alkalinity of sea water caused by photosynthesis and were the first to relate pH changes to carbon dioxide changes in order to measure production of dry organic matter in the sea. Osterhout and Haas (1918a) found the amount of carbon dioxide abstracted by marine algae was a linear function of pH in the range studied by them (pH 8.1 to 8.3). Osterhout and Haas (op. cit.) concluded that the amount of photosynthesis could be measured by the change of pH

values. Moore, Whitley, and Webster in 1921 stated that as the supply of carbon is drawn from dissolved bicarbonates, the reaction of the medium becomes more alkaline and the increase can be used to measure photosynthetic activity. The limit was determined to be when all of the bicarbonate was converted to carbonates at about pH 9.1. After this, free hydroxide ions were formed and the rapid increase in the alkali killed the algae. Schutow (1926) observed that the pH fluctuated when the carbon dioxide of bicarbonate was used up. Saunders in 1926 discussed the dependence of pH on the concentration of carbonate, bicarbonate, and carbon dioxide and on the temperature and neutral salts. Atkins (1926a, b) also discussed temperature, saying that an increase lessens the solubility of carbon dioxide and that a correction was necessary before alkalinity changes could be used for photosynthetic measurements. Atkins (op. cit.) observed annual changes of pH from 7.6 in winter to 9.3 in summer in a fresh-water reservoir and noted that ranges should be greater in fresh-water than in the more highly buffered salt-water. Atkins' method of using pH changes to measure carbon dioxide assimilated by photosynthesis depended upon correlating pH changes by adding .01 N acid with pH changes produced by an equivalent amount of carbon dioxide.

Diurnal pH changes of 7.4 to 9.6 in a Minnesota lake led Philip (1927) to caution investigators against using one pH determination as representative for any natural water. Such large diurnal pH changes might well have encouraged biologists to use this method to measure photosynthesis; however, some other reports gave the opposite impression. In some northeastern lakes of Wisconsin, the maximum diurnal variation was found to be 0.5 pH unit and these lakes ranged, in carbon dioxide of all forms, from 1.0 mg/l to 7.50 mg/l (Juday, et al., 1935). Baumberger and Winzler (1939), using glass electrodes

with a sensitivity of 0.02 pH unit, claimed they could measure carbon dioxide changes with a sensitivity of 2.3 percent.

Little mention had been made concerning diffusion corrections until Osterlind (1947) remarked that it was difficult to measure growth at a pH higher than 8.2 because of carbon dioxide absorption from the air. Osterlind (op. cit.) measured carbon dioxide consumption by pH allowing for alkalinity changes. Ruttner (1948) used conductivity to measure carbon dioxide, employing conversion graphs made by blowing expired air (carbon dioxide) through the medium and graphing the conductivity changes. This same procedure was used later by Verduin (1960) to make pH-carbon dioxide conversion graphs. Verduin (1951, 1956) outlined, in detail, the procedures necessary to use pH changes to measure photosynthetic rates. Verduin (op. cit.) claimed that with modern pH meters, the error involved in measuring carbon dioxide is less than 10 percent even when the pH change is as low as 0.3 pH unit and also that in most cases diffusion was negligible. Ryther (1956) pointed out that the uptake of carbon dioxide was the most direct measure of photosynthesis since the carbon dioxide was equivalent, mole for mole, to organic carbon. Ryther (op. cit.) also stated that the only practical method of following changes in the entire buffer system of an aquatic environment was by pH, but that pH was probably the least sensitive of modern methods.

Recently several authors have been concerned with the proper methods to be used in construction of the pH-carbon dioxide conversion graph. Proper interpretation of the pH-carbon dioxide relationship is necessary for accurate productivity estimates and these recent reports have helped to improve the pH method. Further discussion of these reports is taken up under the section on methods.

Oxygen

Although oxygen is the counterpart of carbon dioxide in the photosynthetic process, oxygen measurements apparently did not receive as much early attention as did pH and carbon dioxide as a method for measuring production. Scott in 1924 studied the diurnal oxygen pulse in a lake, observing definite pulses during clear, calm weather. Scott (op. cit.) noted the oxygen increase to be caused by photosynthesis and absorption and only to photosynthesis after saturation was reached. One of the greatest contributions to the methodology of production biology and to the use of oxygen measurements was the development of the light and dark (L-D) bottle method by Gaarder and Gran (1927). Since that time the L-D bottle method has been used to measure productivity probably more than any other method. Among the first to use the L-D bottle method were Marshall and Orr (1928) who measured the oxygen evolved by diatom cultures in bottles suspended at various depths in the sea.

The L-D bottle method has been subjected to much detailed study in recent years probably as a result of a descrepancy reported occasionally in which the oxygen content of the dark bottle is greater than that of the light bottle. One criticism of the L-D bottle method is that it isolates the photosynthetic organisms from the environment and this point is important in stream studies. Also the L-D bottle method is not useful to measure production by benthic algae and higher aquatic plants. The simplicity and adaptability of the L-D bottle method which gave it much of its popularity may also have deterred development of oxygen methods for use in free-water.

Many investigators, not always interested in productivity, measured the oxygen content of water samples taken from the environment at various time intervals. Wiebe in 1930, working with a fish pond

containing a bloom of Anabaena, found an increase in oxygen of 4.68 mg liter^{-1} to 15.9 mg liter^{-1} from 6:00 AM to 3:00 PM. Wiebe (op. cit.) reported that the oxygen increase in the ponds was roughly proportional to the amount of algae present. Olson (1932) made hourly oxygen measurements in a lake and had data which would have made an excellent diurnal oxygen curve; however, he was only interested in showing the availability of oxygen for fish. Stephenson, Zoond, and Eyre (1934), although they did not use oxygen to measure productivity, did compare the oxygen increases between autotrophic and heterotrophic communities and found that oxygen increases were much greater in the autotrophic communities.

The use of a dropping mercury electrode to produce a continuous oxygen record was reported by Juday, Blair, and Wilda in 1943 thus marking one of the first attempts to place production measurements on a semi-automatic basis. The continuous monitoring of dissolved oxygen by instruments has received considerable attention and development in the past few years. The use of diurnal oxygen measurements as a popular method was greatly advanced when Odum (1956) published, in detail, the procedures for the method as used by him in measuring the productivity of springs in Florida. Free-water gas curves, i.e., diurnal pH-carbon dioxide curves and oxygen curves, may well be the best methods for measuring productivity in shallow or flowing systems, especially when modern instruments are considered (Beyers, et al., 1959).

Artificial Substrates and Pigments

Artificial substrates were first used mostly to collect periphyton or "aufwuchs" for qualitative purposes. Hentschel (1916) is usually credited as the first to employ artificial substrates in aquatic studies and during the 10 to 20 years that followed, most use of artificial substrates was carried out by European biologists.

Much of the work in the United States has been concerned with the adequacy of samples obtained by artificial substrates. Young (1945) claimed that the periphyton composition was governed in part by the type of substrate. Newcombe was one of the first to measure productivity with glass slides and in 1949 and 1950 he reported results claiming that representative groups of periphyton did attach to glass slides and that horizontally placed slides were probably better than those placed vertically. Newcombe (op. cit.) decided the best criteria for exposure time was the time necessary to produce an amount of algae adequate for laboratory analyses. Castenholz (1960) also found glass to be non-selective regarding algal colonization and used a two to four week exposure period for productivity measurements.

The few references cited here are by no means an indication of the amount of work that has been done concerning artificial substrates. Much of this work has little or no bearing on productivity measurements and has not been included here, but may be found in a comprehensive review by Cooke (1956).

The dependence of the photosynthetic process upon chlorophyll led to the use of the optical density or weight of pigment extracts as a measure of production. The early use of pigment extracts was mostly by workers in marine areas. Kreps and Verjbinskaya (1930) were among the first to use this technique. Harvey (1934) contributed considerable information about the use of pigment extracts and for many years the pigment unit developed by him was used. This pigment unit was called the Harvey Plant Pigment Unit (HPPU) and, as expressed by Harvey, was based on acetone extracts of plant pigment per cubic meter of water. Manning and Juday (1941) also gave detailed procedures for using chlorophyll measurements to estimate primary production. Gardiner in 1943 used acetone extracts and at that time pointed out the need for more research into the accuracy and limitations of the pigment method.

Some researchers have tried to correlate pigment extracts with other parameters to test the validity of the pigment method. Juday, Blair, and Wilda (1943) compared chlorophyll extracts with oxygen measurements and with the dry weight of plankton and reported that chlorophyll varied from .39 to 1.06 percent of the dry weight of plankton. Tucker (1949) compared pigment extracts with cell counts and calculated correlation coefficients of .78 to .97 for the pigment and cell count regressions. Grzenda and Brehmer (1960) credited work by Hooper, Ball, and Hayne (ms) as the first to combine artificial substrates and pigment extracts as a method to study productivity. Grzenda and Brehmer (op. cit.) correlated the optical density of phytopigments, extracted from periphyton collected on plexiglass shingles, with the ash-free dry weight of the periphyton. Peters (1959), using the same technique, found that the same regression relationship for phytopigment to organic weight was valid for a number of diatom communities and also stated the possibility of the same relationship being valid for most of the seasons with the exception of the low productivity of winter.

There have been many criticisms of the pigment extract method. Most of the criticisms are concerned with the fact that the relationship of chlorophyll content with organic weight is not consistent, but is influenced by a number of factors such as nutrition, light intensity, species, and age of the algae. Myers and Kratz (1955), studying blue-green algae, reported that there was no relationship between photosynthetic rates and pigment content. Other controversies have been concerned with the proper solvent to use for pigment extraction since the absorbancy spectrum varies with the type of solvent. Until recently the role, in photosynthesis, of pigments other than chlorophyll a has been unknown and many discussions have been concerned over whether or not only chlorophyll a should be used in the pigment method.

Despite the many controversies over the use of the pigment method, it still remains a rapid and attractive method. As Odum (1959) points out, the chlorophyll content of communities appears to be much more consistent than that of individual species and may be an example of "community homeostasis."

C^{14} and Other Methods

The following methods were not employed in this study, but are briefly discussed because of their importance in production studies in general.

Probably the oldest method of measuring production or more likely, standing crop, is that of enumeration and the measuring of volumes of plankton. Enumeration is possible for those algal forms occurring as single cells, thus including most planktonic species and most of the attached diatom flora. Counts are made in Sedgwick-Rafter cells or on membrane filters and reported as number per volume of water. Enumeration fails to give any idea of biomass. The volume of plankton per given volume of water has been used considerably and is measured by concentrating the planktonic forms by centrifugation or by filtration. The accuracy of this method for measuring primary production is lowered by the difficulty of separating zooplankton, detritus, and inorganic particles from the phytoplankton. Studies typifying the early use of enumeration and volumes as methods are those of Kofoid (1903, 1908), Birge and Juday (1914), Weibe (1930), and Ruttner (1930).

One of the most recently developed methods to measure primary productivity is that employing the radioisotope C^{14}. Steeman-Nielsen, in 1952, first described the use of C^{14} in combination with the L-D bottle method as a means of measuring the amount of carbon fixed during

photosynthesis. Since then the C^{14} method has received much attention and critical evaluation. One of the major points has been whether the C^{14} method measures gross or net production or possibly some intermediate value. Doty and Oguri (1959) have made extensive studies of the C^{14} method and probably give the most recently established procedures.

Stream Studies

A recent, extensive review of the ecology of river algae by Blum (1956) makes one point readily apparent, that is, in general river studies have been mostly on plankton and mostly qualitative with only a few quantitative benthic studies. The review presented here has been selected to cover the earlier work and those studies dealing with either production or benthic algae or both.

Kofoid's studies (1903, 1908) of the plankton of the Illinois River are some of the earliest and most noteworthy stream investigations. Kofoid's studies (op. cit.) were very detailed and included the effects of various factors on plankton, measures of the production of plankton in cubic centimeters, and the importance of plankton to fish production. Another quantitative study of river plankton was undertaken on the San Joaquin River in California by Allen (1920). One of the first studies to deal with periphyton rather than plankton was a taxonomic investigation of the "blanket algae" of freshwater pools by Platt in 1916. A study by Eddy (1925) dealt with the description and succession of algal cover on a stream bottom.

Observations of the diurnal gas variations in rivers were made as early as 1928 in England. Oxygen and pH measurements were made continuously over 24 hour periods revealing an oxygen maximum shortly after mid-day and highest seasonal values in the spring along with heavy

diatom growth (Butcher, et al., 1928). Observations of diurnal gas variations such as these probably played a part in the development of diurnal gas curves for the measurement of productivity. The work of R. W. Butcher (1932, 1946), although not primarily concerned with productivity, contributed significantly to the knowledge of periphyton ecology and the use of artificial substrates in streams. Kann (1933) also studied the ecology of periphyton, but in the littoral zone of lakes, and reported the effects of wave action and temperature on algal types.

Whitney (1942) observed the diurnal fluctuation of pH and oxygen in a stream and noted that the peaks of the two occurred together, but he made no use of the data for production estimates. Some recent stream studies have continued to be primarily concerned with taxonomy, distribution, and the general ecology of periphyton (Blum, 1954, 1957, Gumtow, 1955, and Douglas, 1958). But, many of the recent works have dealt with primary productivity. Abdin (1949) measured the number of diatoms per square centimeter that attached to glass slides and Gumtow (1955) measured standing crop of periphyton by number and by volume. The detailed investigations of Odum (1957a, b) initiated the use of diurnal oxygen curves to measure primary productivity in flowing waters. Hoskin (1959) also used diurnal oxygen measurements to estimate the productivity of streams in North Carolina. In a study of a mountain stream, McConnell and Sigler (1959) used relative chlorophyll concentrations and L-D bottle methods to measure the algal productivity. Grzenda (1960) combined diurnal oxygen measurements, periphyton collections on artificial substrates, and chlorophyll extractions to estimate the primary productivity of a warm-water stream in Michigan.

The increase of research on stream productivity indicates a trend which will help to fill an existing gap in our knowledge of stream ecology.

Related Topics and Reviews

There are a number of physical and chemical factors influencing the physiology of aquatic flora and thus affecting photosynthesis and productivity. An immense number of studies have dealt with these factors, both in nature and in cultures. The use of cultures dates back at least to the work of Allen and Nelson in 1910 and the use of algal cultures by plant physiologists and plant biochemists has been very extensive since that time.

Solar radiation, both in quantity and quality, was one of the factors most thoroughly investigated by researchers in the early 1900's. The first studies were concerned with light penetration into water (Pietenpol, 1918, Atkins, 1926a, and Klugh, 1927). Birge and Juday (1929, 1930, 1931, 1932) published a series of reports on the transmission of solar radiation by lake water. Klugh (1930) studied and reviewed the effects of light quality on photosynthesis. Solar energy was studied by Schomer and Juday (1935) regarding production efficiencies at different depths and by Jenken (1937) regarding energy values by spectral quality.

More recent work on light intensity has been undertaken by Dvihally (1960), Kratz and Meyer (1955), Myers (1946), Saito (1958), and Warburg, Schröder, and Gattung (1956). Recent work on light quality has been done by McLeod (1958, 1961), and Warburg and Krippahl (1960) among others.

Temperature effects have usually been studied in cultures and in terms of a Q_{10} and have been reported by many authors including Saito (1958), Talling (1955), Osterhout and Haas (1918), and Van der Paauw (1934).

The biochemical composition of algae has been reported by a number of workers, notably Birge and Juday (1922), Juday (1940), Ketchum and Redfield (1949), Krogh, Lange, and Smith (1930), Schuette (1918), and Spoehr and Milner (1949).

Photosynthesis and nutrient requirements have received so much attention that no attempt has been made here to review these two subjects. Rather, a number of reviews and comprehensive publications are cited here. The subject of algal nutrition is well covered by Barnes (1957), Burlew (1953), Fogg (1953), and Rodhe (1948). Photosynthesis has been reviewed and discussed in detail by such authors as Arnoff (1957), Franck and Loomis (1949), Hill and Whittingham (1955), Rabinowitch (1945, 1951, 1956), and Whittingham (1955).

Other reviews are given by Prescott (1956) on algal ecology, and Saunders (1957) on growth factors of algae. A review by Lund and Talling (1957) on algae and limnological methods is especially comprehensive, as evidenced by 777 references, and Strickland (1960) gives a good account of primary production measurements of marine phytoplankton.

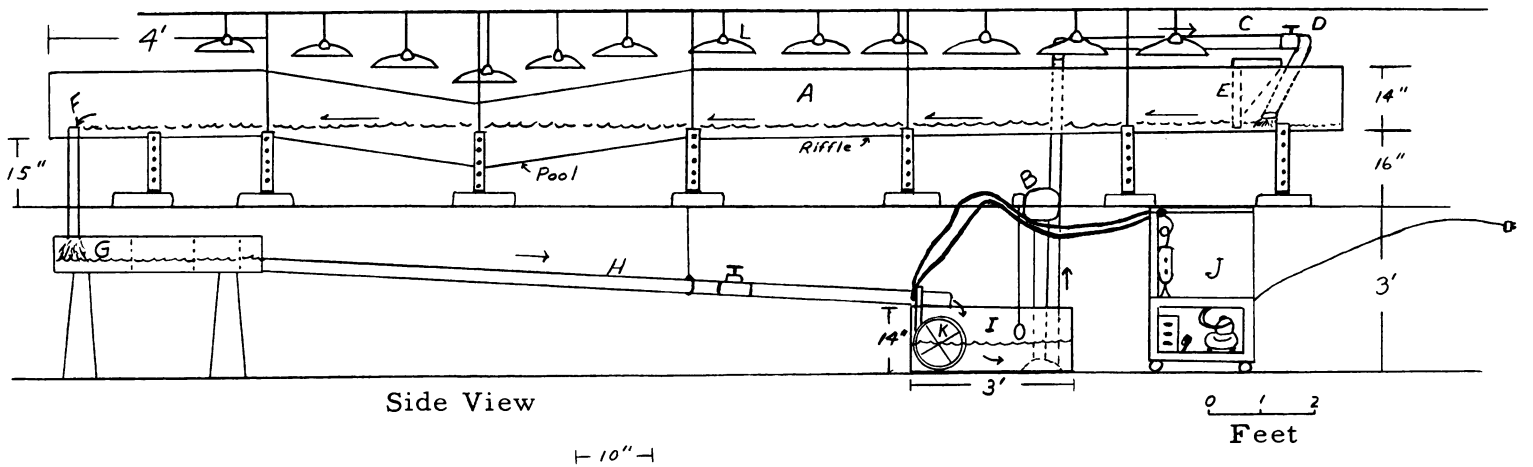
METHODS AND EQUIPMENT

Artificial Streams

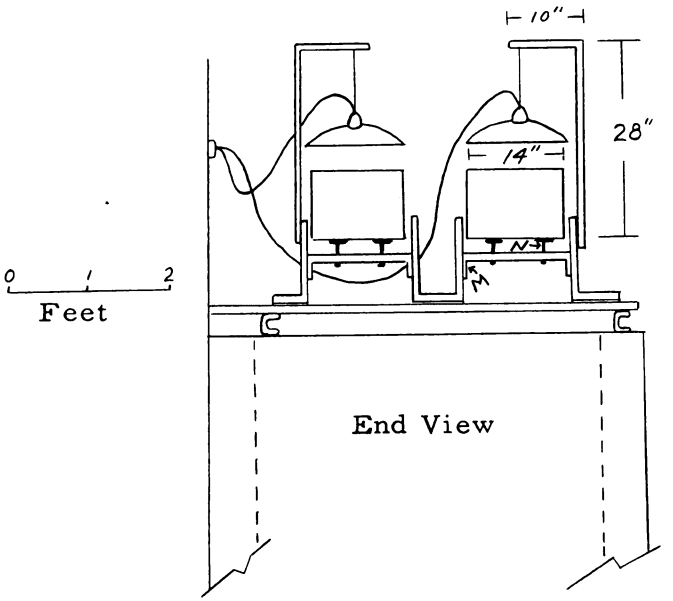
A matched pair of artificial, recirculating streams were used for this study with one of the streams being built to conform to the first, existing stream designed and used by Stokes (1960). The streams were 24 feet long, 14 inches wide, and 10 inches deep and were constructed of six sections of aluminum trough each four feet long (Figure 1). The troughs were lined with clear polyethylene sheeting. The return flow passed from the lower end of the streams, through black polyethylene filter boxes, and by black polyethylene tubing, 1.75 inches in diameter, to the reservoirs located below the troughs. The reservoirs, each with a 300 liter capacity, were also lined with polyethylene sheeting. Identical Homart sump pumps, Model 259.481, were used to pump the water from the reservoirs through polyethylene tubing to the upstream end of the troughs. Plexiglass baffle plates reduced the turbulence of the inflowing water.

The troughs were supported from below, at the union of each section, by threaded bolts inserted in heavy metal cross pieces. The support bolts, with two inch plates welded to the heads, and the metal cross pieces were both movable vertically. Thus the ends of each four foot section could be raised or lowered to create pools or to give the streams the desired gradient. The streams were separate except for valved tubing connections between the return tubes to the reservoirs and between the delivery tubes to the upstream ends. The water of the streams could be intermixed or maintained separately as desired. Valves were also located on the the return tubes and on the delivery tubes making it possible to control the discharge of the streams.

Figure 1. Diagram of artificial streams.



Side View



End View

- Legend -

A. Stream trough	H. Return
B. Pump	I. Reservoir
C. Inlet pipe	J. Cooler
D. Inlet valve	K. Cooling unit
E. Baffle	L. Lights
F. Stream outlet	M. Coarse adjustment
G. Filter	N. Fine adjustment

Modified from Stokes, 1960.

Figure 1

The opaque filter boxes, each with a graded series of aluminum screens, and the reservoirs provided sufficient area for decomposition of organic matter. The lining, filter boxes, and tubing, all of polyethylene, provided chemically inert channels for the water. The few metal exposures, that is, the valves, pump base, and filter screens, were sprayed with clear Krylon plastic paint.

The two streams were covered with clear polyethylene sheeting and the filter boxes and reservoirs were provided with opaque coverings. The loss of water by evaporation and contamination by dust were thus reduced to a minimum.

Nutrient Medium

The streams were each established with 246 liters of distilled water in July, 1959. An inorganic nutrient medium was selected to supply the elements necessary for algal growth. The medium selected was modified from media given by Chu (1942) and by Kratz and Meyer (1955). The composition and concentrations as first added appear in Table 1. The streams were allowed to operate on this medium until May, 1960 when additional nutrients were added (Table 2). As shown in Table 2, other nutrients were also added in July and October of 1960. A nutrient stock solution was used to replace nutrients lost by removal of water samples and algal samples. The stock solution for nutrient replacement contained elements in the following concentrations (milligrams per milliliter): Ca -- 1.02, Mg -- 0.18, Na -- 0.59, N -- 0.93, P -- 0.13, and Si -- 0.25. All nutrients were dissolved in distilled water before being added to the streams.

Within a few days after a nutrient addition the streams reached a steady nutrient state with the nutrient supply recycling by decomposition of algae in the filters and reservoirs. This situation was assumed to

Table 1. The composition of initial nutrient medium in milligrams per liter added to each stream in July, 1959.

Salt	Concentration	Element	Concentration
KNO_3	114	K	47.6
K_2HPO_4	8	N	15.7
$MgSO_4 \cdot 7H_2O$	40	Mg	4.0
$CaCO_3$	40	Ca	16.1
$FeCl_3$	4	Fe	1.8
$Na_2SiO_3 \cdot 9H_2O$	19	Si	1.8
EDTA	41	P	1.4
Microelements*	1 ml liter^{-1}	Cl	2.2
		Na	3.0
		C	4.8
		S	5.2

*Stock solution of micronutrients (grams per liter):

H_3BO_3 -- 2.86

$MnCl_2 \cdot 4H_2O$ -- 1.81

$ZnSO_4 \cdot 7H_2O$ -- 0.22

$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ -- 0.18

$CuSO_4$ -- 0.05

$Co(NO_3)_2 \cdot 6H_2O$ -- 0.49

be comparable to the boil of a spring continuously supplying nutrients. The concentration of nitrate and phosphate, determined by water analyses, was similar to the values reported by Odum (1957b) for some Florida springs.

Ethylene diamine tetra-acetic acid (EDTA) was only used with the first nutrient additions since certain following studies were concerned with effects of chelates.

Table 2. The concentration of elements in milligrams per liter added to each stream on May 24, July 21, and October 7, 1960.

Element	Concentration	
	May 24	July 21 and October 7
Ca	8.0	0.41
Mg	1.8	0.73
Na	4.8	0.24
N	8.1	0.38
P	1.0	0.05
Si	2.0	0.10
Microelements*	-	0.5 ml liter^{-1}

*Micronutrient stock solution as given in Table 1.

Light

Radiant energy was supplied to each stream by a row of 12 incandescent bulbs suspended approximately an equal distance above the stream bottom. Each bulb was equipped with a 14 inch shade reflector. The bulb size and the height of suspension depended upon the particular study. Incandescent bulbs were used because of the predominance of the longer (red) wavelengths over those of fluorescent bulbs having a predominance of the shorter (blue) wavelengths. Although chlorophyll extracts show a higher absorbancy peak in the blue range, the action spectrum of the living cells is greatest in the red range, especially in blue-green algae where phycocyanin absorbs at about 625 mμ (McLeod, 1958, Rabinowitch, 1951). Also, Meier (1932) reported lethal effects of ultra-violet light on a green alga and the use of incandescent bulbs avoids any adverse effects of UV light

more so than would most fluorescent bulbs. The heat from the incandescent bulbs was dissipated before it passed through the polyethylene cover of the streams.

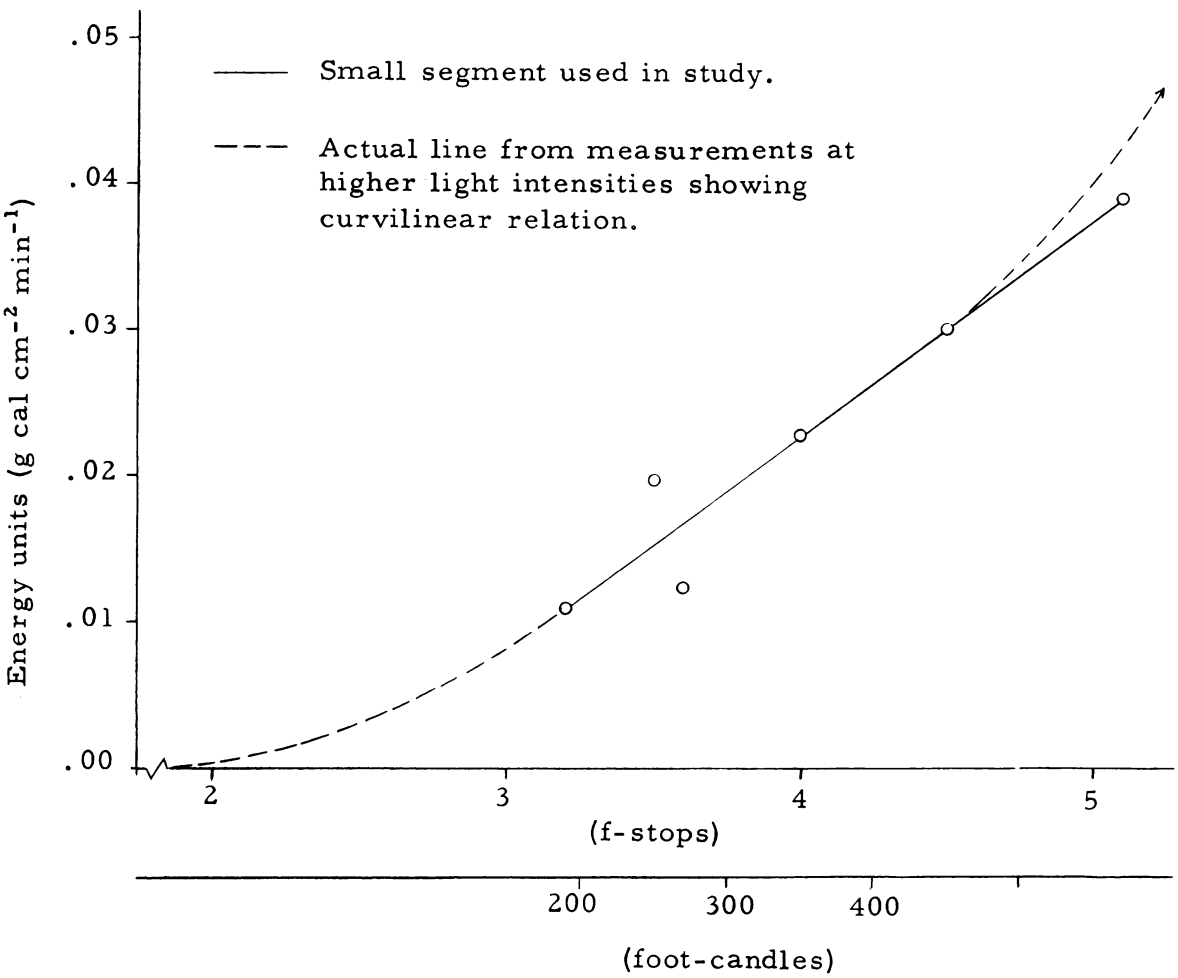
The photoperiod for each stream was controlled automatically by a Paragon Electric Company timer, Model 7008-0 which turned the lights on and off at pre-set times depending upon the particular study.

The amount of light delivered to the streams was determined by two methods. Illumination was measured with a Norwood Super Director exposure meter set for incident readings. The exposure meter was calibrated directly with an Eppley pyrheliometer and a graph was drawn to convert illumination readings to energy units (Figure 2). The linear relationship in Figure 2 is possible for only a small segment of the calibration as the overall relation is curvilinear. Measurements were also made with a Weston Foot Candle Meter, Model 614. Values read in foot candles were converted to energy units using the conversion factor of 1 ft-c = 6.58×10^{-5} g cal cm^{-2} min^{-1} given by Strickland (1958) for the photosynthetic range of wavelengths.

Energy values underwater, at the surface of the algae, were determined directly by the exposure meter wrapped in Dow Saran Wrap and by calculation. Calculations of underwater values were based on surface measurements with the foot candle meter and absorption coefficients ($\underline{k}$) determined from exposure meter readings and from values in the literature for the major wavelengths of incandescent light and distilled water.

Absorption coefficients were calculated for three depths using Lambert's Law, $I = I_0 e^{-kx}$. The values for $\underline{k}$ decreased with increasing depth and probably correspond to those wavelengths that were predominant at that depth (Table 3). The calculated $\underline{k}$ values range from .257 to .0631 at 1.27 cm to 6.35 cm of water depth respectively, corresponding to wavelengths of about 650 mμ (red) to 580 mμ (yellow).

Figure 2. Conversion of exposure meter readings to energy units.



Exposure Meter Readings

Figure 2

This range of wavelengths is in agreement with the spectrum of incandescent bulbs.

Table 3. Absorption of light in artificial streams.

Depth	Absorbancy by Percentage: Artificial streams	Sea Water*	Absorbancy by Absorption coefficient (k): Artificial streams	Wavelengths (mμ) for corresponding k	
0.5 cm	-	21	.257	610**	710***
1.27 cm	28	-	.0934	590	620
6.35 cm	33	-	.0631	570	590
100.0 cm	-	50			

*Richardson, 1933.

**James and Birge, 1938, measurements of monochromatic light in pure water.

***Strickland, 1958, measurements of monochromatic light in pure water.

The conversion of underwater illumination measurements to energy values is based on a report by Dvihally (1960) claiming that, at depths, illumination units and energy units are more proportional for the long wavelengths than for the short wavelengths.

Water Temperature

The water temperature of each stream was regulated by a thermostatically controlled refrigeration unit with the cooling unit located in the reservoir. Temperature was regulated within a range of ± 1.0 C and was recorded by a Taylor thermograph for weekly periods alternately

in the two streams. Water temperatures were also checked periodically with a hand thermometer. The water temperature was measured each time a water sample was taken for oxygen analysis.

Water Velocity and Discharge

The velocity of flow and discharge were controlled by adjusting the inlet valves and the gradient of the streams.

The best estimate of flow velocity was calculated from discharge measurements and the stream dimensions. The actual discharge was measured with a volumetric container and a stop watch at the reservoir outfall. The product of stream width and depth divided into the discharge gave the average velocity at the point where the dimensions were measured.

Specific Conductivity and Total Solids

The amount of dissolved materials in the streams was primarily a result of the salts added as nutrients and the recycling of elements from the decomposition of algae.

Electrical resistance was measured with a Model RC-7 conductivity meter manufactured by the Industrial Instruments Company. Readings were corrected to 18 C and expressed as the reciprocal of resistance, i.e., micromohs per centimeter.

Total solids were measured gravimetrically as described for total residue in "Standard Methods for the Examination of Water, Sewage and Industrial Wastes" (APHA, AWWA, FSIWA, 1955).

Alkalinity, pH, and Carbon Dioxide

Alkalinity, pH, and carbon dioxide are interrelated and, in the artificial streams, were primarily a function of the calcium and magnesium that were added as nutrients and of the buffer system. The addition of calcium and magnesium combined with atmospheric carbon dioxide diffusing into the water forms the carbonate-bicarbonate system as follows:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

$$Ca(HCO_3)_2 \rightleftharpoons Ca^{++} + 2HCO_3^-$$

$$HCO_3^- \rightleftharpoons CO_3^{=} + H^+$$

$$CO_3^{=} + H_2O \rightleftharpoons HCO_3^- + OH^-$$

It can be seen from the formulas that as carbon dioxide enters the system more hydrogen, bicarbonate, and carbonate ions are formed. When calcium and magnesium ions are present they combine with the bicarbonate and carbonate ions until an equilibrium is established. The alkalinity and pH thus depend largely upon the carbon dioxide tension and the availability of combinable cations.

Alkalinity was determined by the method described by Welch (1948). The pH was measured with a Beckman Model H-2 pH meter. The concentration of free carbon dioxide was determined by nomograph making use of measurements of pH, alkalinity, total solids, and temperature (APHA, AWWA, FSIWA, 1955).

Phosphorus

Phosphorus was added equally to both streams as one of the nutrient elements. Phosphorus concentrations in the streams were monitored for two reasons: 1) the role of phosphorus as a nutrient is important, and 2) the concentration of phosphorus was probably as low

as any of the major elements in the streams, as is often the case in nature.

Concentrations of total and dissolved phosphorus in water samples were determined by the ammonium molybdate-stannous chloride method given by Ellis, Westfall, and Ellis (1948). Samples for total phosphorus were digested while those for dissolved phosphorus were treated directly with the color-producing reagents before being read in a Klett-Summerson colorimeter using a red filter.

The concentration of cellular phosphorus in algal samples was determined to provide a measure of the phosphorus metabolism of the algae. Organic phosphorus of prepared algal samples was determined using the same procedures as for total phosphorus. The samples of algae were ashed in a muffle furnace and weighed prior to the phosphorus analysis.

Nitrogen

Nitrogen was also added equally to both streams as one of the nutrient elements. Nitrogen was determined in water samples and algal samples for the same reasons as was phosphorus.

The concentration of available nitrogen ($NH_4^+ + NO_2 + NO_3$) in water samples was measured by the reduction method while the organic nitrogen of algal samples was determined by the micro-Kjeldahl procedure; both methods being described in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes" (APHA, AWWA, FSIWA, 1955). Color was produced by nesslerization in the reduction method and readings were made with a Klett-Summerson colorimeter using a green filter. Boric acid and titration with 0.02 N H_2SO_4 were used for the organic nitrogen. Algal samples were oven dried at 55 C and weighed prior to organic nitrogen analysis.

Calorimetry

It was necessary to know the caloric value of algal samples to calculate energy efficiencies, to compare the energy content of algae grown in different areas and under different conditions, and to gain some insight into the composition of the algae.

Duplicate samples of algae were analyzed for each caloric determination. The measurements were made with a Parr Oxygen Bomb Calorimeter, plain type, series 1300, using the method described in "Oxygen Bomb Calorimetry and Combustion Methods" (Parr Instrument Company, 1960). All algal samples were oven dried at 55 C, powdered, pelleted, and weighed in preparation for the analysis.

Weights

All gravimetric measurements were made on an analytical balance and were recorded when two successive readings of ± 0.5 mg were made. Dry weight was determined after drying in an oven at 55 C. Ash-free dry weight, assumed to be organic weight, was measured after ignition of the sample at 550 C in a muffle furnace. All weighings were made after the samples had cooled to room temperature in a dessicator.

Primary Productivity

The rate of energy transformation from light energy into organic matter was measured by three methods which are discussed in the following paragraphs. Three methods were used as a comparative study in an effort to determine the most satisfactory method for use in flowing waters. In the final comparisons, all values were converted to calories.

Diurnal Oxygen Curves

The amount of oxygen evolved by the algae during photosynthesis was estimated using the upstream-downstream method given by Odum (1956). The upstream station was located just before the first incandescent bulb and the downstream station was located under the last bulb. The difference between the oxygen concentrations of the upstream and the downstream stations was a measure of net production for the stretch of water under the lights. Respiration was automatically subtracted. Since there were no springs or seepage, no oxygen could be added by drainage accrual. It was necessary to correct for diffusion using the formula given by Odum (op. cit.).

$$D = KS$$

where D = the diffusion rate per area (g O_2 m^{-2} hr^{-1}),

K = the gas transfer coefficient at 0% saturation (g O_2 m^{-2} hr^{-1}),

and S = the saturation deficit between water and air.

Efforts to calculate the gas transfer coefficient, K, produced inconsistent results, so values for K were estimated from tables given by Odum (op. cit.) for comparable water conditions.

The estimation of gross productivity requires the measurement of respiration. Respiration was estimated from oxygen concentrations measured after the lights were turned off using the corresponding diffusion corrections.

All estimations of production and respiration were calculated in volume dimensions, g O_2 m^{-3} hr^{-1}, and were converted to area dimensions, g O_2 m^{-2} hr^{-1}, by dividing by the mean water depth between the stations. The conversion to area measurements was done to facilitate comparison with data from artificial substrates already on an area basis.

Three estimates of productivity, in g O_2 m^{-2} day^{-1}, were made from the oxygen data. These were gross productivity for the area under the lights, net productivity for the same area, and net productivity for the entire stream including the filter and reservoir. Net productivity for the entire system required the estimation of respiration and B. O. D. in those stream areas in darkness. Respiration and B. O. D. were determined by reversing the oxygen calculations of the upstream-downstream method and correcting for diffusion. It was thus assumed that the difference in oxygen concentration between the downstream station and the upstream station was due to respiration and B. O. D. when the water passed through the filter and reservoir.

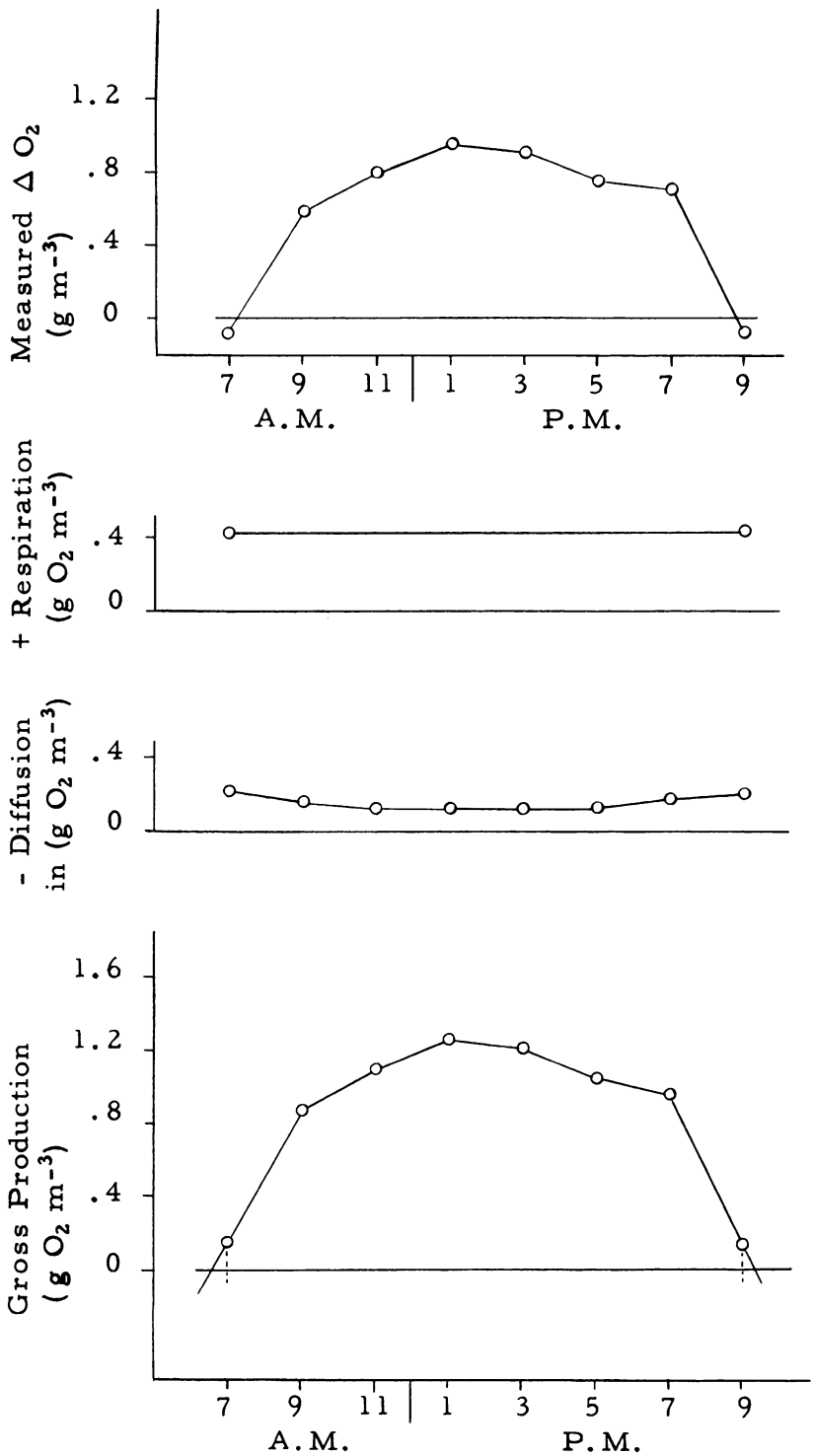
A typical oxygen curve is given in Figure 3 and a sample calculation of productivity is given in Table 4. It was found, as expected, that the area under the curve agreed with calculated estimates.

All oxygen concentrations were determined by the Alsterberg (azide) modification of the Winkler method as given in "Standard Methods for the Examination of Water, Sewage, and Industrial Wastes" (APHA, AWWA, FSIWA, 1955). Oxygen saturation values for use in diffusion corrections were corrected for temperature, atmospheric pressure, and vapor pressure. Water samples for oxygen determinations were taken from the same water mass as it passed from the upstream to the downstream stations.

Diurnal pH-Carbon dioxide Curves

The uptake of carbon dioxide by algae during photosynthesis was measured as an estimate of productivity. Measurements were made indirectly by recording pH changes and converting these changes into carbon dioxide concentrations using the method described by Verduin (1951, 1956). Conversion graphs (Figure 4) were made stepwise, by bubbling respired air through water samples and back-titrating with 0.02 N NaOH (Verduin, 1960).

Figure 3. Rate of oxygen production in stream No. 2 during the temperature study, on June 28, 1960, as estimated by the area under the curve method.



Area under curve equals 13.0 g O_2 m^{-3} day^{-1}

Figure 3

Table 4. Production rate of oxygen in stream No. 2 as calculated from measurements taken on June 28, 1960 during the temperature study.

Time	(A) Oxygen change ($g\ m^{-3}hr^{-1}$)	(B)* Diffusion ($g\ m^{-3}hr^{-1}$)	(C = A+B) Oxygen corrected for diffusion ($g\ m^{-3}hr^{-1}$)	(D = C+R)** Oxygen corrected for respiration ($g\ m^{-3}hr^{-1}$)	Gross production ($g\ m^{-3}day^{-1}$
7 AM	-0.08	-0.21	-0.29	0.14	
9	0.60	-0.15	0.45	0.88	
11	0.80	-0.13	0.67	1.10	$\overline{D}$ = 1.081,
1 PM	0.96	-0.13	0.83	1.26	1.081x12 hrs.
3	0.92	-0.13	0.79	1.22	= 12.98
5	0.76	-0.13	0.63	1.06	
7	0.72	-0.18	0.54	0.97	
9	-0.08	-0.20	-0.28	0.15	

*Diffusion is based on a gas transfer coefficient, K, of 1.75 $g\ m^{-3}\ hr^{-1}$ at 0% saturation, e.g., the saturation deficit at 7 AM = 12% and (B) = 1.75 x 12 = -0.21.

**R is respiration and is based on measurements taken at night and corrected for diffusion. R for this estimate equals 0.43 $g\ m^{-3}\ hr^{-1}$.

Net production = Gross production - R = 12.98 - (0.43 x 12 hrs.)
= 7.84 $g\ m^{-3}\ day^{-1}$.

Figure 4. Titration curves of stream water for conversion of change in pH units to μmoles of carbon dioxide.

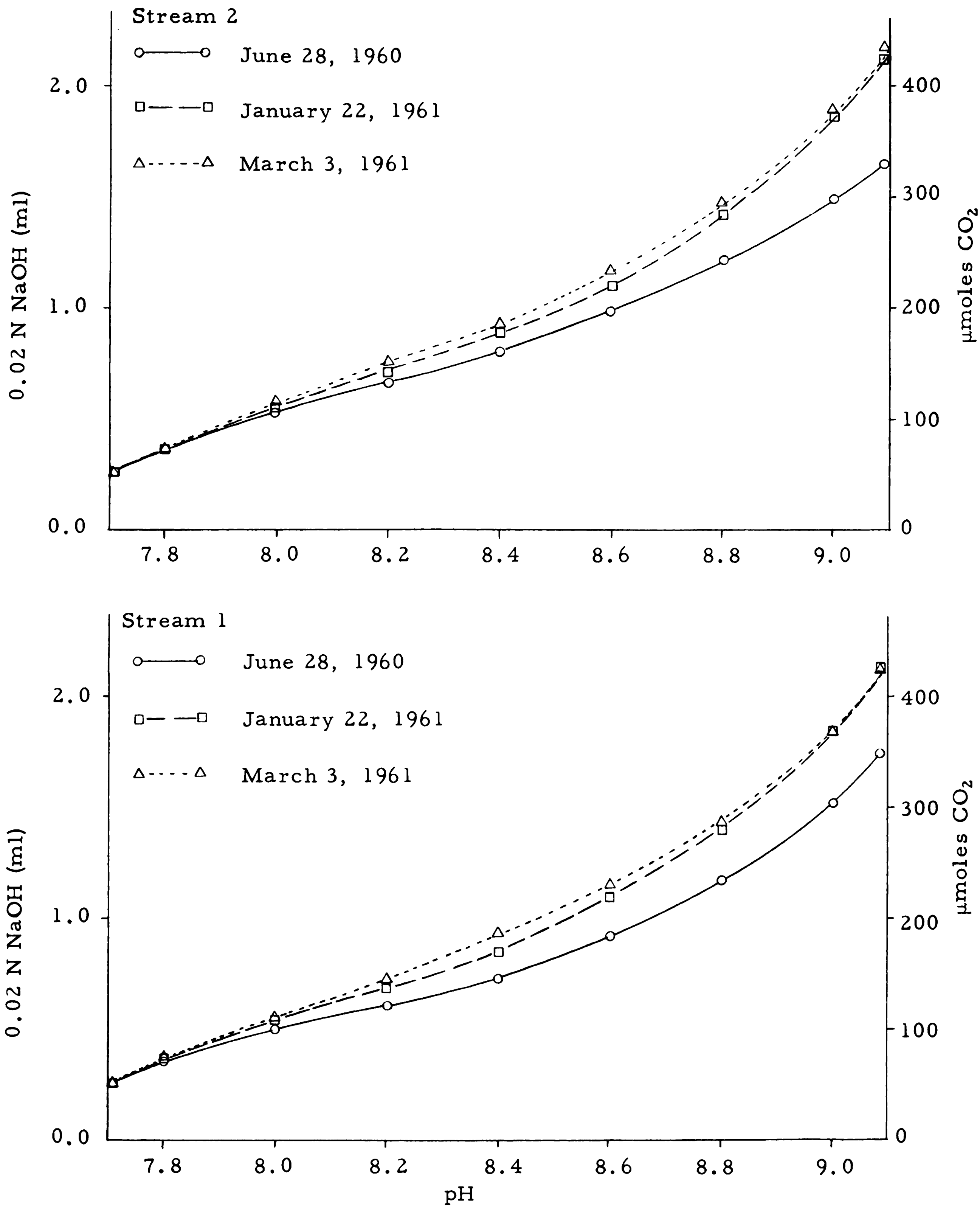

Stream 2
June 28, 1960
January 22, 1961
March 3, 1961
0.02 N NaOH (ml)
μmoles CO_2
0.0
1.0
2.0
0
100
200
300
400
7.8
8.0
8.2
8.4
8.6
8.8
9.0
Stream 1
June 28, 1960
January 22, 1961
March 3, 1961
0.02 N NaOH (ml)
μmoles CO_2
0.0
1.0
2.0
0
100
200
300
400
7.8
8.0
8.2
8.4
8.6
8.8
9.0
pH

Figure 4

A number of criticisms have been made about the methods used to construct the pH-carbon dioxide conversion graphs. An attempt was made to interpret and avoid these criticisms as much as possible. It has been pointed out that curves made by titrating with different solutions, i.e., acids, bases, and CO_2 water, diverge from each other except in the range above pH 7.8 where the agreement is good (Beyers and Odum, 1960, Verduin, 1960). The range of pH change in the artificial streams was primarily from pH 7.9 to 9.0. Lyman (1961) pointed out that titrations with solutions altered the alkalinity of the sample and thus produced an error. If the titration is done stepwise using several samples, each one for a small range of the titration curve, then the amount of titrant used for any one sample is slight and the titrant does not accumulate enough to cause an appreciable error (Verduin, 1961). In the single step titration the titrant accumulates enough to change the alkalinity during the latter part of the titration.

Figure 4 shows the conversion curves for different dates to deviate slightly and demonstrates the value of preparing a curve for each productivity estimate. Only three curves for each stream were used in this study with the curve of the closest date and water conditions being used for the particular productivity estimate.

Diffusion of carbon dioxide into or out of the water was considered to be negligible. Beyers and Odum (1959) state that no diffusion correction is ordinarily needed with the pH-carbon dioxide method. Verduin (1956) states that even at maximum invasion rates, the air-water phase boundary is an effective barrier to carbon dioxide. The conditions of pH, alkalinity, temperature, and total solids in the artificial streams were such that the range of free carbon dioxide in the water was from 0.1 to 1.5 mg $liter^{-1}$. At the lowest pH (7.8) a small amount of carbon dioxide may have left the system, while at the highest pH (9.0) a small amount may have entered the water.

The pH was found to increase throughout the day and to decrease throughout the night, thus the maximum change could be recorded by measurements made at the beginning and end of the daily photoperiod. The maximum pH change was converted by graph to micromoles of carbon dioxide.

The measurement of carbon dioxide uptake is an estimation of net productivity. Gross productivity is calculated by adding respiration to the net productivity. The diurnal carbon dioxide curves revealed respiration at night to be equal to net productivity during the day (Figure 5). Thus gross productivity was twice net productivity.

Carbon dioxide estimates were made on the same days as the oxygen measurements. Samples for pH were taken at the lower end of each stream.

Periphyton Growth and Pigment Extraction

The third method used to measure net productivity was the gravimetric analysis of periphyton collected on artificial substrates. A fourth method, phytopigment analysis, was also tested, but it gives the same values as the periphyton growth upon which it is based. The phytopigment method is therefore an alternate method to be used after correlations between periphyton weight and phytopigment concentration have been established.

Plexiglass shingles, with an exposed area of 1.5 square decimeters, were placed on the stream bottoms at five designated stations. Periphyton colonized the shingles and was allowed to grow on these substrates for a period ranging from 12 to 28 days. The substrates remained in the streams for the longer periods when the periphyton growth was slowest. A pair of substrates from each station gave a total of 10 used for each estimate of productivity for each stream. The accumulated growth from the paired substrates was combined to produce a more

Figure 5. Diurnal carbon dioxide curve prepared from pH measurements in stream No. 2 during the temperature study on July 5, 1960.

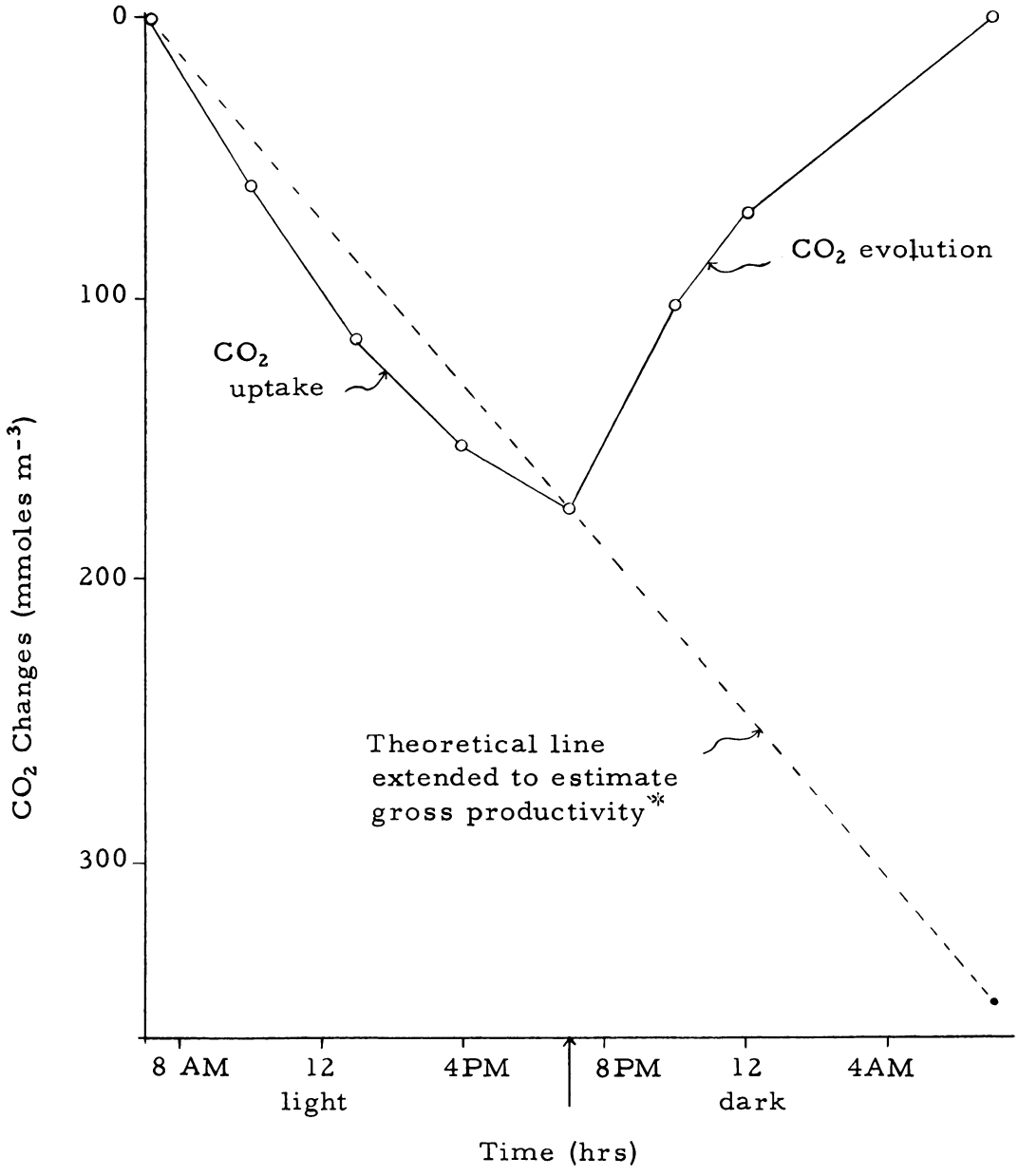


Figure 5

*Net productivity = 175 mmoles CO_2 m^{-3} day^{-1} or 7.7 g CO_2 m^{-3} day^{-1}. Assuming day respiration equals night respiration, then extended line (dashes) is gross productivity (350 mmoles CO_2 m^{-3} day^{-1}).

representative sample for each measurement. Calculations based on data collected by Stokes (1960) revealed growth from 63 paired substrates to have a mean coefficient of variation of 16.3% with a range from 0 to 140%.

A procedure was used allowing measurement of both total weight and phytopigment concentration from the same sample. The periphyton was scraped and rinsed from the substrates into 95% ethanol and allowed to stand in darkness for at least 48 hours. Filtration through a membrane filter (pore size = 0.45 mμ) separated the periphyton from the ethanol-soluble phytopigments. The phytopigment extract was adjusted to 50 ml and the concentration measured with a Klett-Summerson colorimeter using a red filter (640 - 700 mμ). After the phytopigment reading, the extract was recombined with the periphyton in an evaporating dish, dried at 55 C, and the constant weight determined on an analytical balance. Ash-free dry weight, assumed to be organic weight, was then determined after ignition of the sample at 550 C.

The periphyton weight in grams was divided by the number of days that the substrates remained in the streams, less a six day colonization period, to give net productivity in g m^{-2} day^{-1}. The amount of algae that grew on the substrates during the colonization period was too small to be measurable. Phytopigment concentrations were corrected for a deviation from the Lambert-Beer Law and reported as phytopigment units as described by Grzenda and Brehmer (1960). Calculated regressions allowed conversion of phytopigment units to organic weight of periphyton.

The conventional use of a wavelength of 660 mμ or a light passing through a red filter to measure the absorbancy of phytopigment extracts is based on the absorbancy peak of chlorophyll a. Chlorophyll a is believed to be the main pigment involved in the transfer of light energy and is a major pigment in all of the algal groups thus providing the basis

for its use as a standard. The absorbancy peak at 660 mμ is fairly well isolated from peaks of other pigments except for chlorophyll b in which the absorbancy peak is about 645 mμ (Strickland, 1960). The influence of phycocyanin, with a peak at 615 mμ, may have a slight effect on the absorbancy reading especially if a wide wave band is used. It is assumed here that the influence of phycocyanin in the pigment investigations will have little or no bearing on comparisons made with other pigment studies other than to compensate for chlorophyll b, present in the Chlorophyceae, and chlorophyll c in diatoms (peak at 630 mμ), both of which are reported to be lacking in the blue-green algae (Strickland, op. cit.).

STANDARD CONDITIONS AND STUDY PROCEDURES

Many of the physical characteristics of the artificial streams were controlled and altered for the various studies that were undertaken. A description of these characteristics in the standard and study conditions is a necessary prelude to the presentation of results.

The standard conditions of a characteristic were in effect in both streams except when that characteristic was being studied (Table 5). The characteristics studied were temperature, photoperiod, light intensity, current velocity, and chelates. Other relevant characteristics, not specifically studied, are shown in Figures 6 and 7. The characteristic altered for study was maintained in most cases for at least one month. Diurnal oxygen and pH measurements were usually made for each stream at the end of the second and fourth weeks. Substrates were removed at the same two week intervals, except during the current velocity study. The growth pattern of the algae was also studied during the current velocity study. For the growth pattern the substrates were removed from the streams in such a manner that growth measurements were made by two or three day increments from 3 to 34 days. After each study period the streams were maintained under standard conditions for at least two weeks to return the two streams to an equal productivity level.

The velocity of flow as given in Table 5 appears to be very slow when compared to natural streams. The highest velocity recorded was 4.2 cm sec^{-1} in the riffle area of stream 1. Odum and Hoskin (1957), in a study of a laboratory stream microcosm, referred to velocities of 8 to 20 cm sec^{-1} as high and those of 2 cm sec^{-1} as low velocities and explained that these were the velocities occurring very near (2.5 mm) the algae and thus correspond to much higher velocities in a natural system.

Table 5. Standard and study conditions in the artificial streams.

Characteristic	Study Condition		Standard Condition*
	Stream 1	Stream 2	
Temperature (degree C)	20.0	25.6	23.9
Stream Velocity ($cm\ sec^{-1}$)			
pool	0.6	0.1	0.5
Riffle	4.2	0.6	2.5
Radiant Energy ($g\ cal\ m^{-2}\ day^{-1}$)			
at water surface	63,000	136,800	68,200
at algae surface	44,200	95,000	48,240
Photoperiod (hours)	4 on - 4 off	12 on - 12 off	12 on- 12 off
Chelate ($mg\ liter^{-1}$)	30	10	trace

*The standard condition of a characteristic was in effect at all times except when that characteristic was being studied, e.g., the streams were always at 23.9 C except during the temperature investigation when the study temperatures were in effect. Standard conditions were the same for both streams.

Figure 6. Specific conductivity and total alkalinity throughout the study period.

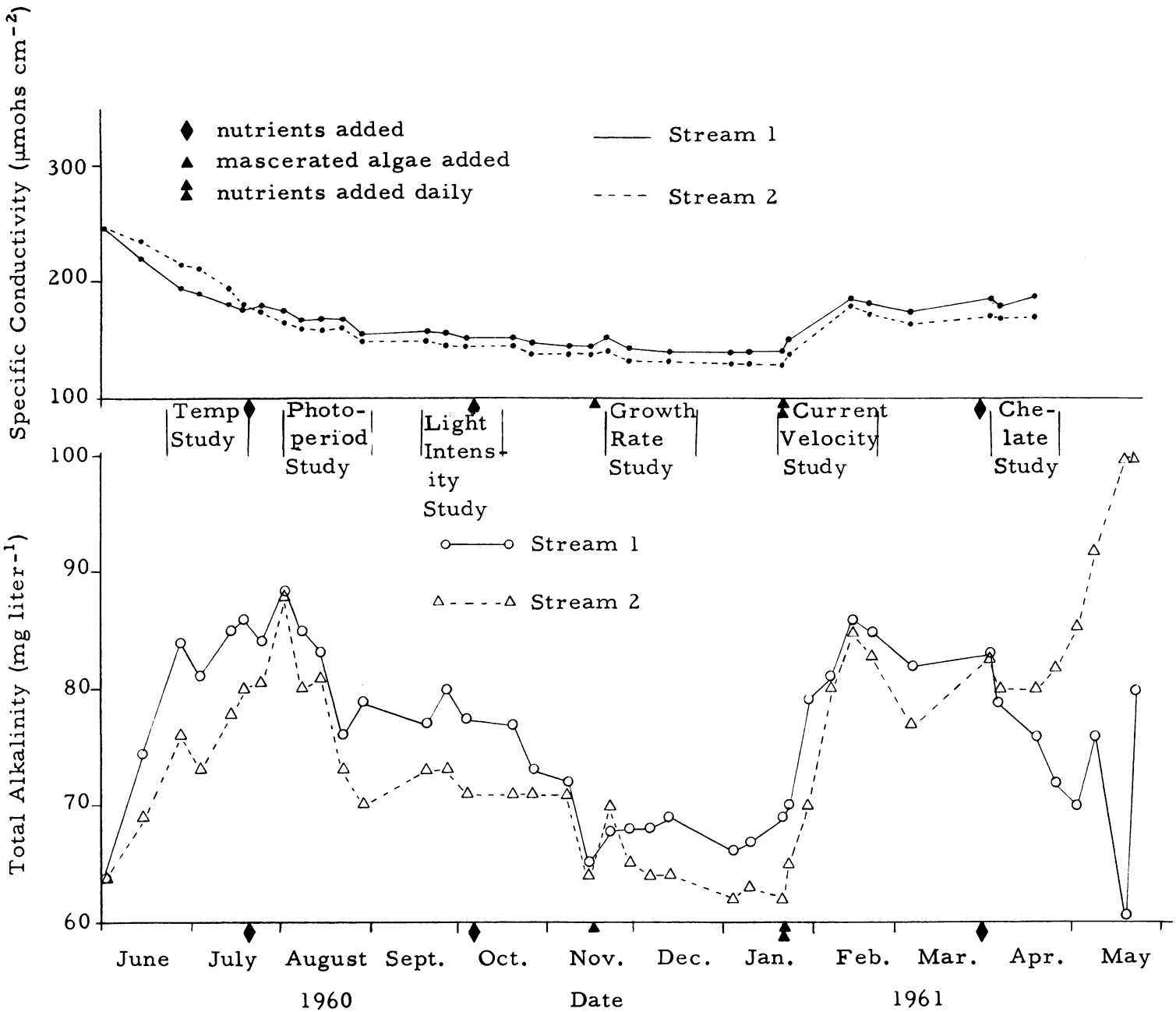

Specific Conductivity (μmohs cm⁻²)
nutrients added
mascerated algae added
nutrients added daily
Stream 1
Stream 2
300
200
100
Temp Study
Photo-period Study
Light Intensity Study
Growth Rate Study
Current Velocity Study
Chelate Study
Total Alkalinity (mg liter⁻¹)
100
90
80
70
60
Stream 1
Stream 2
June
July
August
Sept.
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
1960
Date
1961

Figure 6

Figure 7. Available nitrogen and total phosphorus throughout the study period.

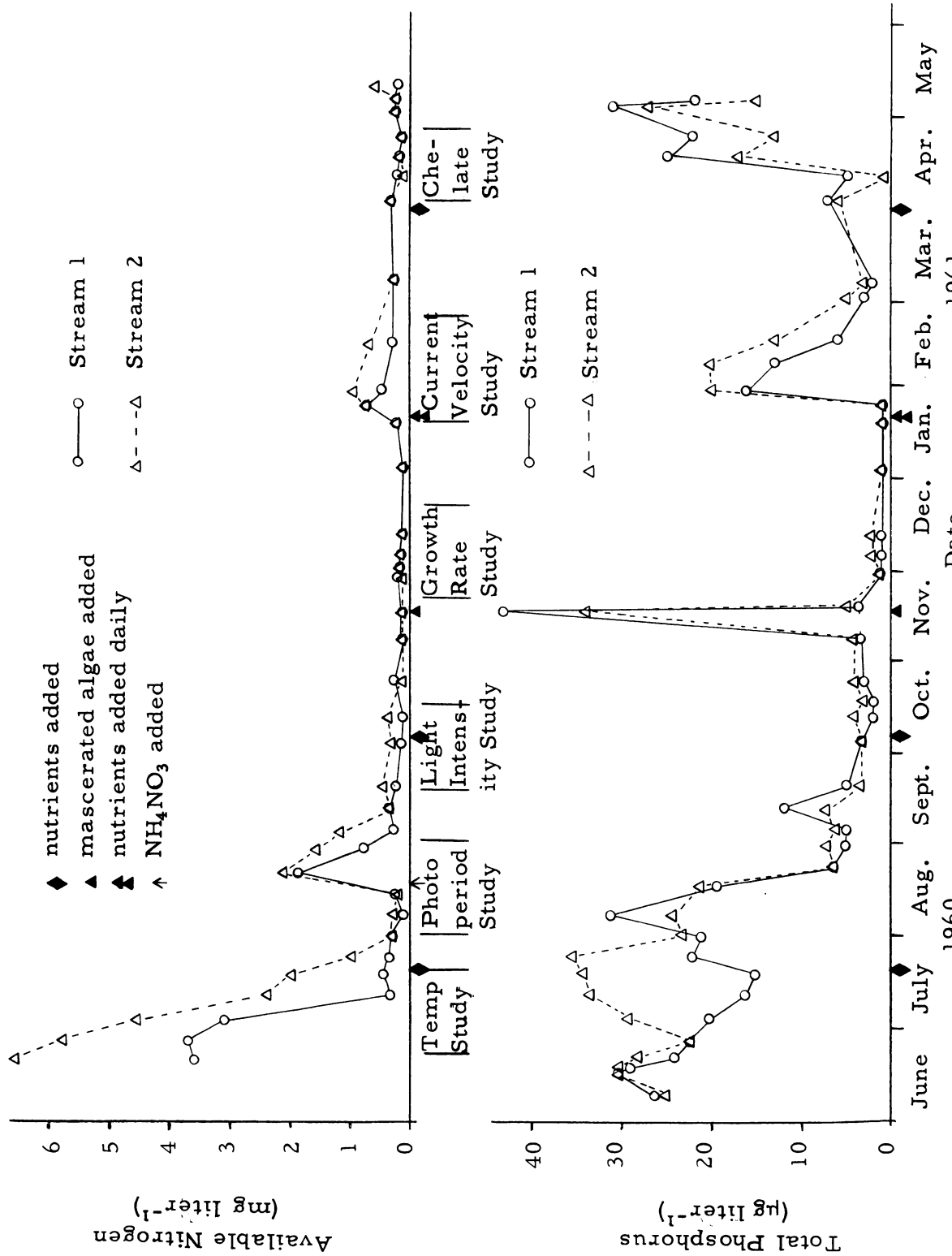
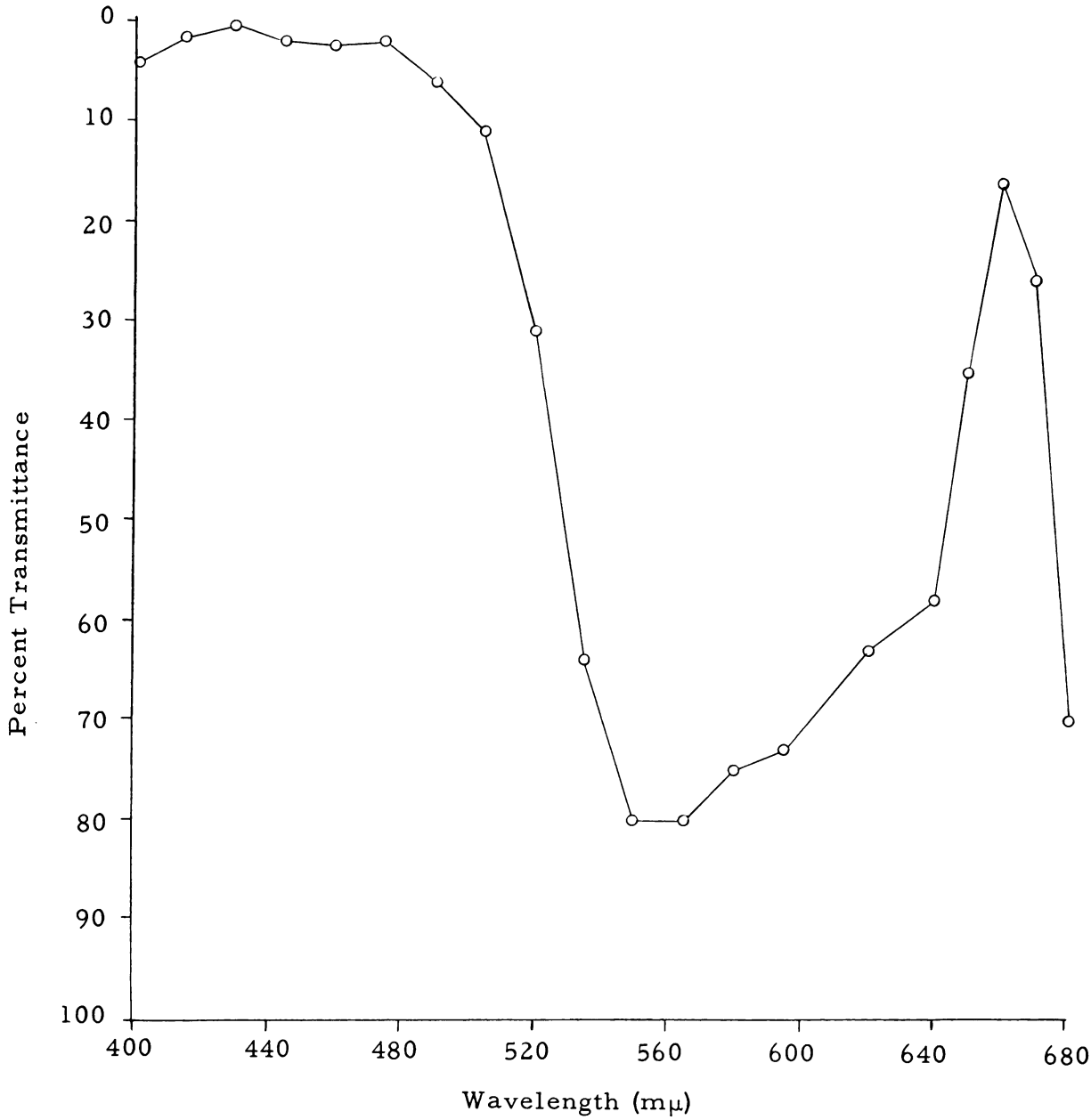

nutrients added
mascerated algae added
nutrients added daily
NH_4NO_3 added
Stream 1
Stream 2
Available Nitrogen (mg liter^{-1})
Total Phosphorus (µg liter^{-1})
Temp Study
Photo period Study
Light Intensity Study
Growth Rate Study
Current Velocity Study
Chelate Study
June
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan.
Feb.
Mar.
Apr.
May
1960
1961
Date

Figure 7

The artificial streams were established in the summer of 1959 and used for pilot studies until the first study was initiated in the summer of 1960. This time also served as a period for colonization, algal succession, and equalization of the two streams at a steady state. The streams were seeded by introducing a few periphyton-covered stones taken from a local warm-water stream, the Red Cedar River. Colonization required only a few weeks and the first periphyton was a mixture of green and blue-green algae, and diatoms. Succession moved toward a climax species and a steady nutrient state. The first alga to become dominant was Palmellococcus sp. Chodat, an ellipsoidal, green alga, which after a few months gave way to a blue-green alga, Plectonema Boryanum Gomont, identified by Dr. G. W. Prescott. This small, filamentous alga represented the climax species and was dominant throughout the remainder of the study. Odum (1957a) states where environments become extreme for any reason the number of species decreases. The artificial streams, like Silver Springs studied by Odum (op. cit.), were extreme in being stable and this accounts for the paucity of algal species. The genus Plectonema is described by Prescott (1951) as becoming yellow or brown with age. The mat of algae in the artificial streams turned to a drab yellow-brown color sometime after P. Boryanum became dominant. Nitrogen at that time was at a low concentration. New growth of this algal mat was a dark green color and was apparent in numerous tufts about the periphyton mat and on the artificial substrates. This aging process has been described by Kingsbury (1956) and Odum and Hoskin (1957) for Plectonema nostocorum Bornet ex Gomont. Kingsbury (op. cit.) decided the yellowing condition resulted from a depletion of nitrogen in the medium and believed photosynthesis to be stopped or much reduced in the yellow state. Odum and Hoskin (op. cit.) reported that photosynthesis did not cease entirely when the yellow-brown condition was present. The magnitude of gas exchange in this study also indicated that

photosynthesis by P. Boryanum in the yellow-brown stage did not cease entirely. The absorbancy spectrum of new growths of P. Boryanum is given in Figure 8 and the peaks agree well with those given by Kingsbury (1956) for the pigments present in healthy P. nostocorum.

Figure 8. Absorbancy spectrum of 95% ethanol extract of Plectonema Boryanum Gomont.



Pigment wavelengths*
Chlorophyll a: 430, 660
C-phycocyanin: 615, 650

B carotene	450
Myxoxanthophyll	480
Myxoxanthin	490
	520

Figure 8

*Kingsbury, 1956

RESULTS AND DISCUSSION

Effects of Study Conditions

Temperature

The effect of water temperature on the rate of primary production was determined by creating a temperature difference of 5.6 C between the two artificial streams, holding other characteristics equal, and comparing the net productivity of the two streams. Stream 2 was maintained at the higher temperature (25.6 C) and was expected to have the higher rate of production. An increase in temperature, at least up to an optimum, will increase most metabolic processes, but not all at the same rate.

The results of most temperature studies have been reported in terms of Van't Hoff's quotient (Q_{10}), the number of times that the rate of a process increases with a 10 C rise in temperature. The Q_{10} value generally accepted for photosynthesis is about 2.0 while the range for respiration is about 2.0 to 2.5. Most enzymatic reactions have a Q_{10} of 1.4 to 2.0. Several authors have reported Q_{10} values for algae in the same approximate temperature range as was used in this study. Osterhout and Haas (1918b) found Q_{10} values for Ulva of 1.69 for photosynthesis and 2.04 for respiration. Van der Paauw (1934) studied several green algae and reported, at 15 to 25 C, a Q_{10} range of 1.7 to 2.1 for carbon dioxide assimilation and a range of 1.7 to 2.4 for respiration. Talling (1955) reported a Q_{10} of 2.3 for the growth rate of a planktonic diatom and a Q_{10} of 2.2 was given for photosynthesis by freshwater phytoplankton by Ichimura and Aruga (1958).

Measurements by carbon dioxide and substrates revealed the net production to be highest in the warmer water of stream 2, while oxygen measurements showed the opposite (Figure 9). Statistical analysis of the values in Figure 9, using a one-tailed Student's "t" test for matched pairs, indicated no significant difference between production at the higher temperature and production at the lower temperature at the 5% level; however, the higher temperature production was significantly greater at the 10% level ($t = 1.537 > t_{.90} = 1.48$, $df = 5$).

The mean estimates of net productivity in g cal m^{-2} day^{-1} for the two temperatures were used to calculate the Q_{10} value. A logarithmic relationship was assumed and the following formula was used.

$$Q_{10} = \left(\frac{P_1}{P_2}\right)^{\frac{10}{t_1 - t_2}}$$

where $P_1 = 715.1$, the productivity at temperature t_1 (25.55 C) and $P_2 = 595.7$, the productivity at temperature t_2 (20.0 C). Thus $Q_{10} = 1.20^{1.801} = 1.4$. A value of 1.4 for net productivity indicates that respiration increased approximately the same as or slightly more than photosynthesis. The effect was probably due to an increase in enzymatic reaction rates.

The average net productivity or rate of energy transfer for the two streams was 595.7 g cal m^{-2} day^{-1} for stream 1 and 715.1 g cal m^{-2} day^{-1} for stream 2. Gross productivity for stream 1 was 1451.0 g cal m^{-2} day^{-1} and for stream 2 was 1735.4 g cal m^{-2} day^{-1}.

Photoperiod

The effects of the length of photoperiod on primary productivity were investigated by maintaining the lights of stream 1 on a "four hours on - four hours off" basis while the lights of stream 2 were on continuously for 12 hours and off for 12 hours. Thus both streams received

Figure 9. The effect of temperature on net production in artificial streams as measured by carbon dioxide, oxygen, and substrates.

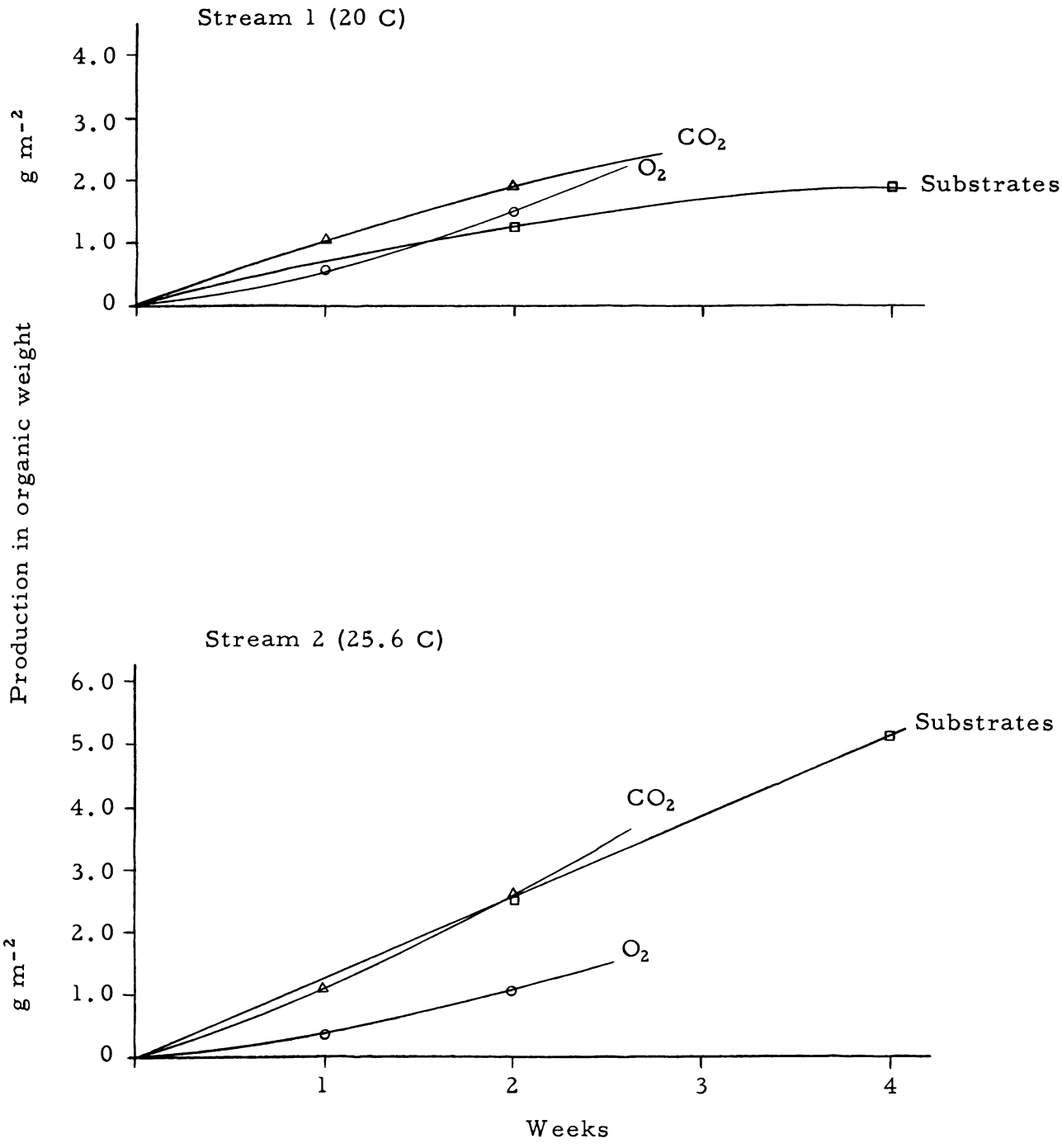

Stream 1 (20 C)
4.0
3.0
2.0
1.0
0
CO_2
O_2
Substrates
Production in organic weight
g m^{-2}
Stream 2 (25.6 C)
6.0
5.0
4.0
3.0
2.0
1.0
0
Substrates
CO_2
O_2
g m^{-2}
1
2
3
4
Weeks

Figure 9

the same amount of radiant energy daily, but at different intervals. Black crepe paper was draped over the streams to isolate the photoperiod conditions. Other physical and chemical characteristics of the two streams were equal.

It is known that energy fixation by photosynthesis is more efficient under conditions of flashing light than during continuous light. This is due to the occurrence of certain dark reactions. It is doubtful that dark reactions, which enhance production under flashing light, would be important enough to influence the production rate under the conditions of this study. However, Steeman-Nielsen (1960) has shown with C^{14} studies that 1 to 3% of light saturation fixation occurs in the dark if the period is about four hours. Apparently the dark fixation is rapid immediately after the lights go off and then declines. If this is the case one might expect some increase in productivity by breaking up the daily dark period even if only into three of four intervals.

The results of the photoperiod study are given in Figure 10. Measurements by carbon dioxide and substrates show production to be slightly higher under the divided photoperiod of stream 1. Oxygen measurements were approximately equal for the two streams. The data of Figure 10 were subjected to a one-tailed matched pairs test. The analysis showed no significant difference at the 5% level between the productivity of stream 1 and that of stream 2 ($P = .30$). The statistical analysis suggests that little effect can be at attributed to the length of photoperiod as long as the daily quantity of light received is equal for both streams.

The mean rate of net energy transfer for stream 1 was 691.3 g cal m^{-2} day^{-1} and for stream 2 the same rate was 649.9 g cal m^{-2} day^{-1}. The gross energy transfer for streams 1 and 2 was 2222.1 and 2005.6 g cal m^{-2} day^{-1} respectively.

Figure 10. The effect of photoperiod on net production in artificial streams as measured by carbon dioxide, oxygen, and substrates.

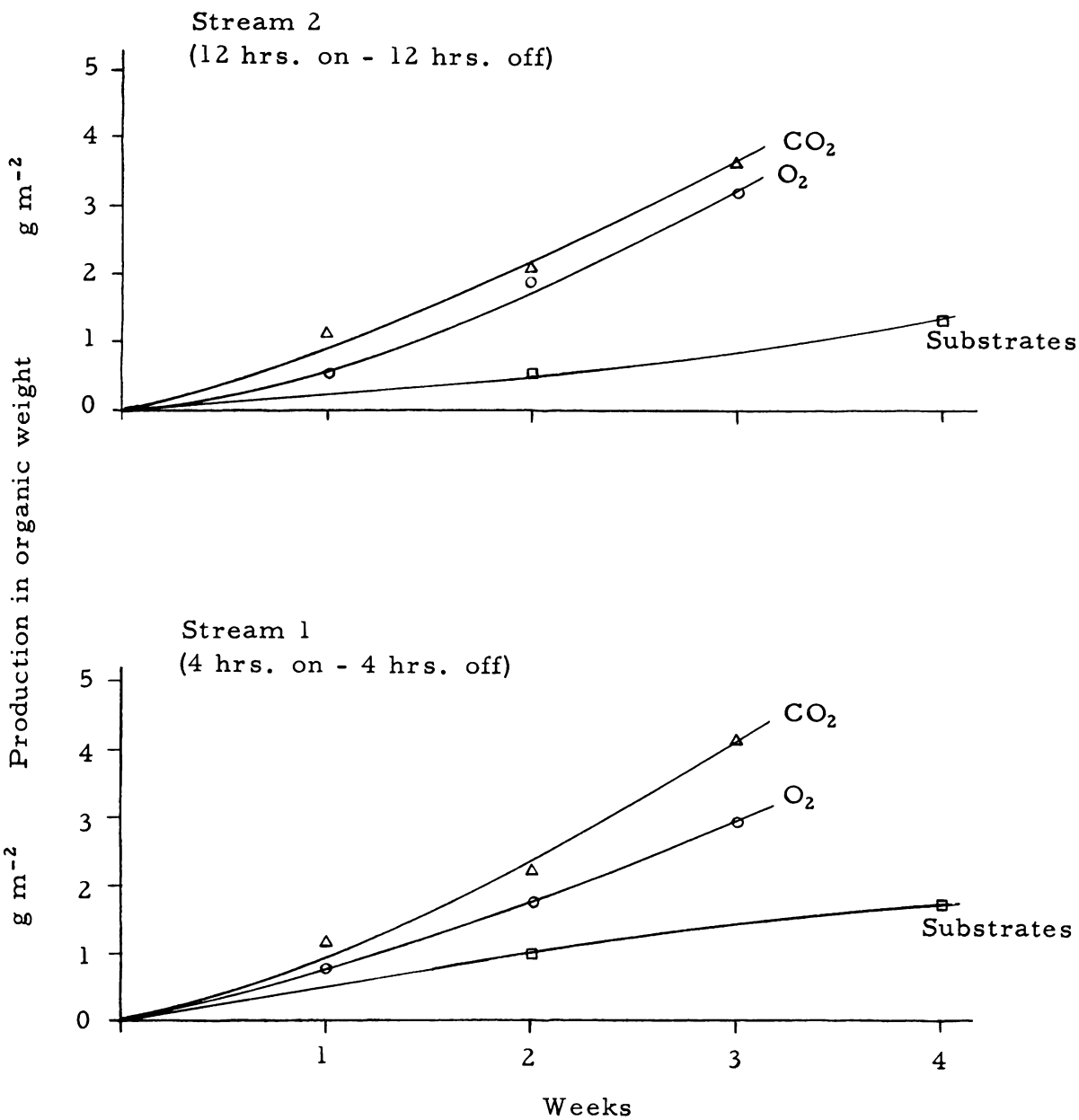

Stream 2
(12 hrs. on - 12 hrs. off)
5
4
3
2
1
0
CO_2
O_2
Substrates
Production in organic weight
g m^{-2}
Stream 1
(4 hrs. on - 4 hrs. off)
5
4
3
2
1
0
CO_2
O_2
Substrates
g m^{-2}
1
2
3
4
Weeks

Figure 10

Light Intensity

The light intensity provided for the two streams was changed from the standard conditions so a difference of over two-fold existed between the streams. The effect of the difference in light intensity was then determined. Stream 1 received the lower incident intensity of 63,000 g cal m^{-2} day^{-1} (120 ft-c) provided by 100 watt bulbs suspended about 17 inches above the stream bottom. Stream 2 received 136,800 g cal m^{-2} day^{-1} (276 ft-c) from 150 watt bulbs suspended about 12.5 inches above the stream bottom. The incident energy was slightly greater in the pool areas of both streams since the lights were lowered to compensate for water depth.

The radiant energy received by stream 2 was about 1% of the average daylight value (1.92 g cal cm^{-2} min^{-1}) and about 12% of the optimum photosynthetic value of 0.1 to 0.15 ly min^{-1} (720,000 to 1,080,000 g cal m^{-2} day^{-1}) given by Strickland (1960). Ichimura and Aruga (1958) noted that light saturation values varied with the major groups of algae, being highest for blue-greens, but at the same time light saturation was at a low of 372 ft-c when influenced by oligotrophic waters. Kratz and Meyer (1955) reported growth rates to increase rapidly when light was increased from 100 to 180 ft-c and to increase more slowly when light was further increased to 260 ft-c. A report on Chlorella cultures by Myers (1946) gave 35 ft-c as the intensity for the greatest photosynthetic capacity, probably referring to efficiencies, and 100 ft-c as the intensity for the greatest growth rate. It is apparent from these reports in the literature that the effect of the range of radiant energy used in this study should result in an increase in productivity under the higher energy rate.

The difference in production attributed to light intensity is given in Figure 11. Measurements by carbon dioxide and oxygen show production to be higher under the higher light intensity of stream 2.

Figure 11. The effect of light intensity on net production in artificial streams as measured by carbon dioxide, oxygen, and substrates.

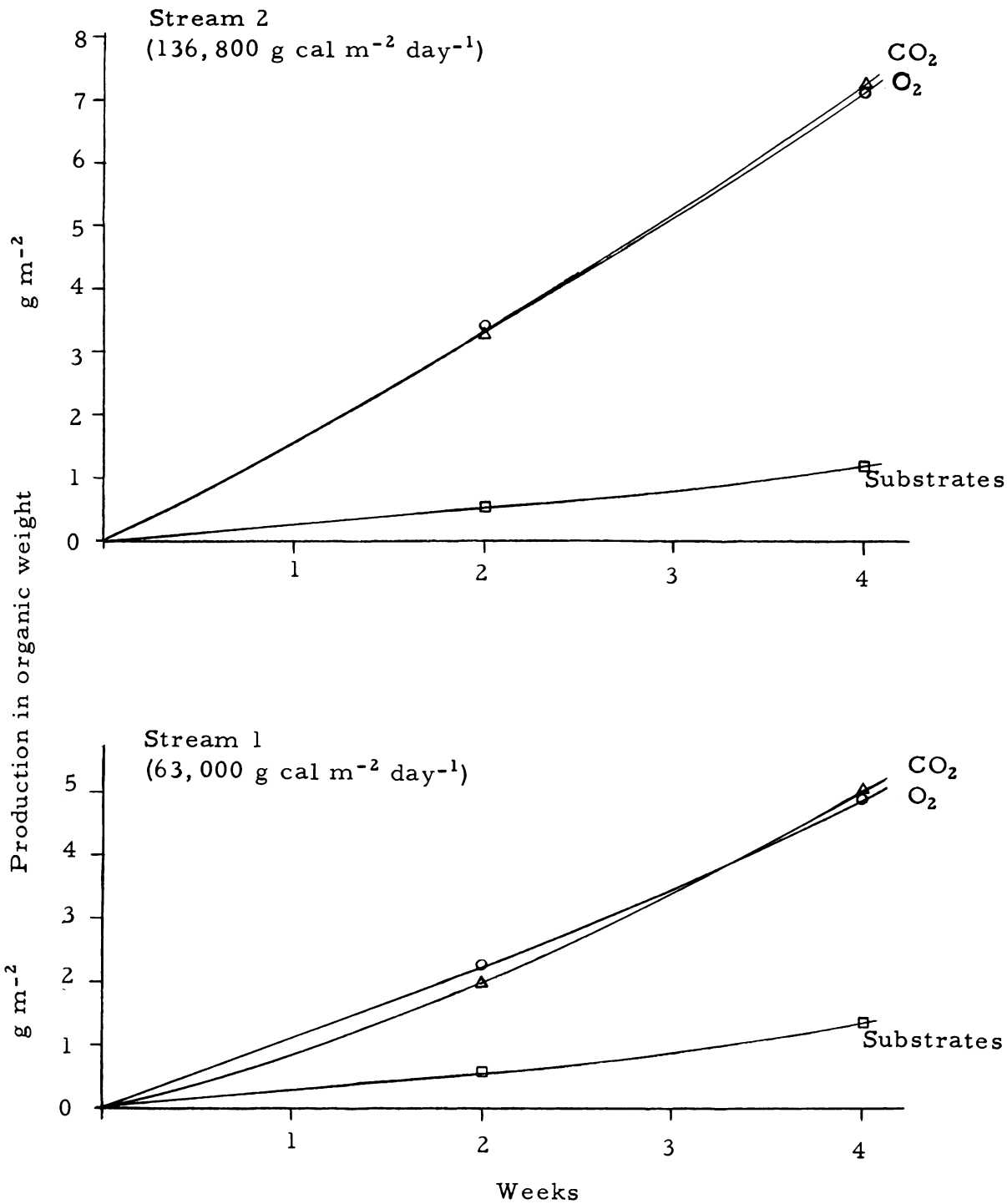

Stream 2
(136, 800 g cal m-2 day-1)
CO2
O2
Substrates
Stream 1
(63, 000 g cal m-2 day-1)
CO2
O2
Substrates
Production in organic weight
g m-2
g m-2
Weeks

Figure 11

Data from substrates show little difference between the two streams. The production of stream 2 was significantly greater than that of stream 1 at the 2.5% level as demonstrated by a one-tailed matched pairs test ($t = 2.674 > t_{.975} = 2.571$, $df = 5$).

The net productivity of stream 1 was 869.9 g cal m^{-2} day^{-1} and of stream 2 was 1259.5 g cal m^{-2} day^{-1}. Gross productivity of stream 1 was 2504.4 g cal m^{-2} day^{-1} compared to 3380.5 g cal m^{-2} day^{-1} for stream 2.

Since only two light intensities were used it is not possible to indicate whether the relationship of light intensity to productivity is linear or hyperbolic as discussed by Strickland (1960); however, considering the low light intensities of this study relative to saturation values in the literature, it might be assumed that the relation here is linear.

Current Velocity

The effect of flowing water on productivity and on other biological phenomena is one of the major differences between streams and lakes. The belief that water movement does influence productivity, directly or indirectly, is one of the main objections against using the L-D bottle method to measure the production of flowing waters. Reports that flowing water enhances production have been largely limited to recent years. Earlier reports were mostly secondary observations such as that made by Butcher (1946), who noted that the number of algal cells per square millimeter of glass slide was higher where the current was fast than where it was slow. Gessner and Pannier (1958) studied the respiration of Anabaena in Warburg flasks and learned that maximum respiration was reached at a much lower oxygen tension when the flasks were agitated than when the flasks were still. Some recent studies have been specifically directed at determining the current effect.

Whitford (1960) theorized that an increase in current produced a steeper diffusion gradient thus facilitating a better exchange of materials between the cells and the environment. Whitford and Schumacher (1961) reported that carbon dioxide evolution by algae was 70% greater in current than in still water. Rawstron (1961) collected evidence showing that the growth rate of periphyton on artificial substrates was greater in riffles than in pools in the Red Cedar River.

Evidence of the effect of flow on productivity was acquired in two ways in this study. Both streams had a riffle and a pool area where the velocity of flow differed. Under standard conditions the velocity of the pools was 0.5 cm sec^{-1} while the riffle velocity was 2.5 cm sec^{-1}. Comparisons of growth on artificial substrates at the different stations was used to indicate any effect of the current. The velocity of flow over the surface of the substrates in the riffle area was actually greater than the riffle area in general since the substrate thickness reduced the average water depth. The velocity of water over the substrates was approximately 3.4 cm sec^{-1} rather than the 2.5 cm sec^{-1} in the general riffle area.

The dry weight of periphyton collected on artificial substrates was used to measure the effect of current on production. Substrates were located at all five stations in both streams and data from all studies were included in the results. Stations 1 and 2 represent the riffle areas and stations 3 and 4 are from the pools. Station 5 is not representative of either area since light intensity at station 5 was less than the other stations. The dry weights from the five stations were subjected to a one-way analysis of variance and the results, given in Table 6, show the production of stations to be significantly different at the 5% level. Kramer's modification (1956) of Tukey's multiple range test revealed the production of stations 1 and 2 to be significantly different from the remaining stations in both streams. Stations 1 and 2 were not significantly

Table 6. Analysis of variance of production in dry weight at the five stations of streams 1 and 2.

Stream 1

Source of Variation	Sum of Squares	df	Mean Square	F Ratio
Stations	52,345.55	4	13,086.39	$F = \frac{13,086.39}{1,301.54} = 10.054$
Within	206,944.30	159	1,301.54	$F_{.95}(4, 159) = 2.43$
Total	259,289.85	163		

Station means in milligram dry weight per substrate:

1	2	3	4	5
49.21	56.18	23.96	20.11	8.92

Kramer's multiple range test reveals stations 1 and 2 to be significantly different from stations 3, 4, and 5, but not from each other.

Stream 2

Source of Variation	Sum of Squares	df	Mean Square	F Ratio
Stations	58,280.18	4	14,570.04	$F = \frac{14,570.04}{1,538.94} = 9.467$
Within	238,535.35	155	1,538.94	$F_{.95}(4, 155) = 2.43$
Total	296,815.53	159		

Station means in milligrams dry weight per substrate:

1	2	3	4	5
60.94	50.08	24.91	21.43	9.07

Kramer's multiple range test reveals stations 1 and 2 to be significantly different from stations 3, 4, and 5, but not from each other.

different from each other and stations 3, 4, and 5 were not significantly different from one another.

Before the higher production of the riffle areas can be attributed entirely to current effect it is necessary to rule out the effects of nutrients and light intensity. Light measurements and calculations showed that the light intensity at the substrate surfaces, although not constant for all studies, was slightly greater in the pool than in the riffle areas. This was a result of placing the lights slightly nearer the stream bottom in the pool areas to compensate for the greater water depth to be penetrated by the light waves. It is assumed that the slight difference in light quality reaching the substrates as a result of the different water depths did not influence production enough to alter the conclusions.

Water samples for nutrient analysis were ordinarily taken from only one site in the streams; however, on a few occasions samples for phosphorus were taken from riffle and pool sites to determine if upstream periphyton was receiving the benefit of greater phosphorus concentrations. The results of eight paired samples from each stream for total phosphorus were analyzed by a two-tailed matched pairs test. Results indicated no significant difference at the 5% level between the phosphorus concentrations of riffle and pool sites in either stream (stream 1: $P = .10$, stream 2: $P = .30$). It is thus assumed that phosphorus concentrations did not influence production in the riffle sites more than in the pool sites.

Since the phosphorus concentration was as low as any other major nutrient, it is assumed that the concentrations of other nutrients did not decrease enough downstream to limit production in the pool. The higher production of the upstream riffle areas is thus attributed to greater current velocity.

Other evidence that water velocity influences production was collected in a manner similar to those described for the first studies. The gradient of the two streams was changed so stream 1 had a velocity

faster than the standard conditions and the velocity of stream 2 was slower than standard conditions (Table 5). Production was measured by carbon dioxide, oxygen, and substrates and all three methods demonstrated higher production in the faster stream (Figure 12). The data were analyzed using a one-tailed matched pairs test and the results indicated that production in stream 1 was greater than production in stream 2 at the 5% level of significance ($t = 2.295 > t_{.95} = 2.015$, $df = 5$).

The mean net and gross productivity in g cal m^{-2} day^{-1} for stream 1 were 1994.2 and 6026.5 respectively while the same productivity values for stream 2 were 1485.3 and 4356.5.

Chelate

Ethylene diamine tetra-acetic acid (EDTA) was used to determine the effects of organic chelation on primary productivity. The action of chelates is due to a strong affinity of the organic molecules for polyvalent metallic ions. When EDTA is added to a nutrient medium, polyvalent metallic ions such as iron, magnesium, and calcium are complexed with the chelate. The basis for suspecting an increase in productivity when chelates are added is that certain insoluble nutrients, required for algal growth, are complexed and brought into solution, while other nutrients tied to the insoluble forms are then indirectly released into solution. This applies to iron and phosphorus. When iron is oxidized from the ferrous to the ferric state, as occurs in oxygenated water, it becomes insoluble and when phosphate is present the two elements precipitate as insoluble ferric phosphate (Hutchinson, 1957). It has been demonstrated that ferric iron precipitated in bottom muds can be brought into solution by complexing with an added chelate (Schelske, 1962). When the ferric phosphate bond is broken the soluble phosphate ion is freed. Both iron and phosphorus are necessary for the growth of algae. In this study phosphorus availability was emphasized although it is

Figure 12. The effect of current velocity on net production in artificial streams as measured by carbon dioxide, oxygen, and substrates.

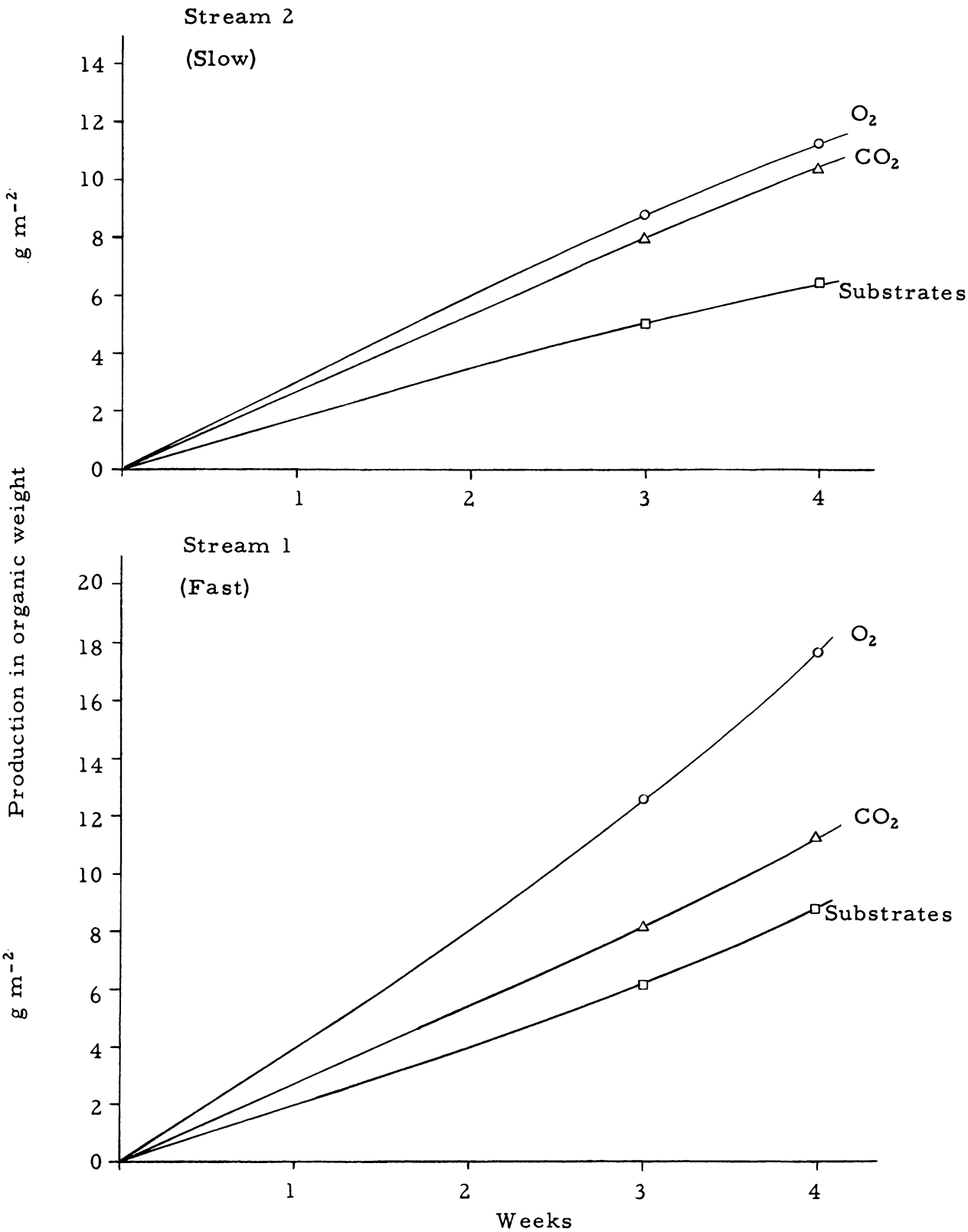

Stream 2
(Slow)
O_2
CO_2
Substrates
Stream 1
(Fast)
O_2
CO_2
Substrates
Production in organic weight
g m^{-2}
g m^{-2}
Weeks
0
2
4
6
8
10
12
14
16
18
20
1
2
3
4

Figure 12

assumed that any increase in productivity could be attributed to increase in available iron as well as to phosphorus, since algae have the capacity to use chelated iron. It has been speculated that many metallic nutrients are complexed in aquatic systems by naturally occurring organic chelates. Fogg and Westlake (1955) stated that the large amounts of cellular products, lost or secreted by blue-green algae, may act as chelates.

The effect of chelate additions on the phosphorus concentrations of the artificial streams was investigated during the first year when the streams were being colonized and established. EDTA was added to each stream at a concentration of 40 mg liter^{-1}. Soluble phosphorus concentrations were then compared to prechelate concentrations. The response to the chelate was indicated by a rapid rise in the soluble phosphorus concentration of both streams (Figure 13). At the end of 56 hours the total soluble phosphorus release in streams 1 and 2 was 25.83 mg and 32.47 mg respectively. The source of the released phosphorus was assumed to be from previously precipitated compounds of iron and other metallic elements, possibly calcium or magnesium. It is evident that the chelate effect on phosphorus release was significant.

The effect of EDTA on the primary productivity of the artificial streams was studied approximately 16 months later. The chelate was again added to both streams, but stream 2 received a concentration of 30 mg liter^{-1} and stream 1, 10 mg liter^{-1}. It was expected that stream 2 would show a higher productivity than stream 1. All methods of estimating productivity confirmed the expectation (Figure 14). The production values were submitted to a matched pairs analysis using a one-tailed test. The results of the statistical analysis indicated that production in stream 2 was greater than in stream 1 at the 2.5% level of significance ($t = 2.876 > t_{.975} = 2.365$, $df = 7$). The mean net and gross productivity in g cal m^{-2} day^{-1} for stream 1 were 612 and 1636 respectively and for stream 2 were 1096 and 2236 respectively.

Figure 13. Action of a chelate on orthophosphate concentration in artificial streams.

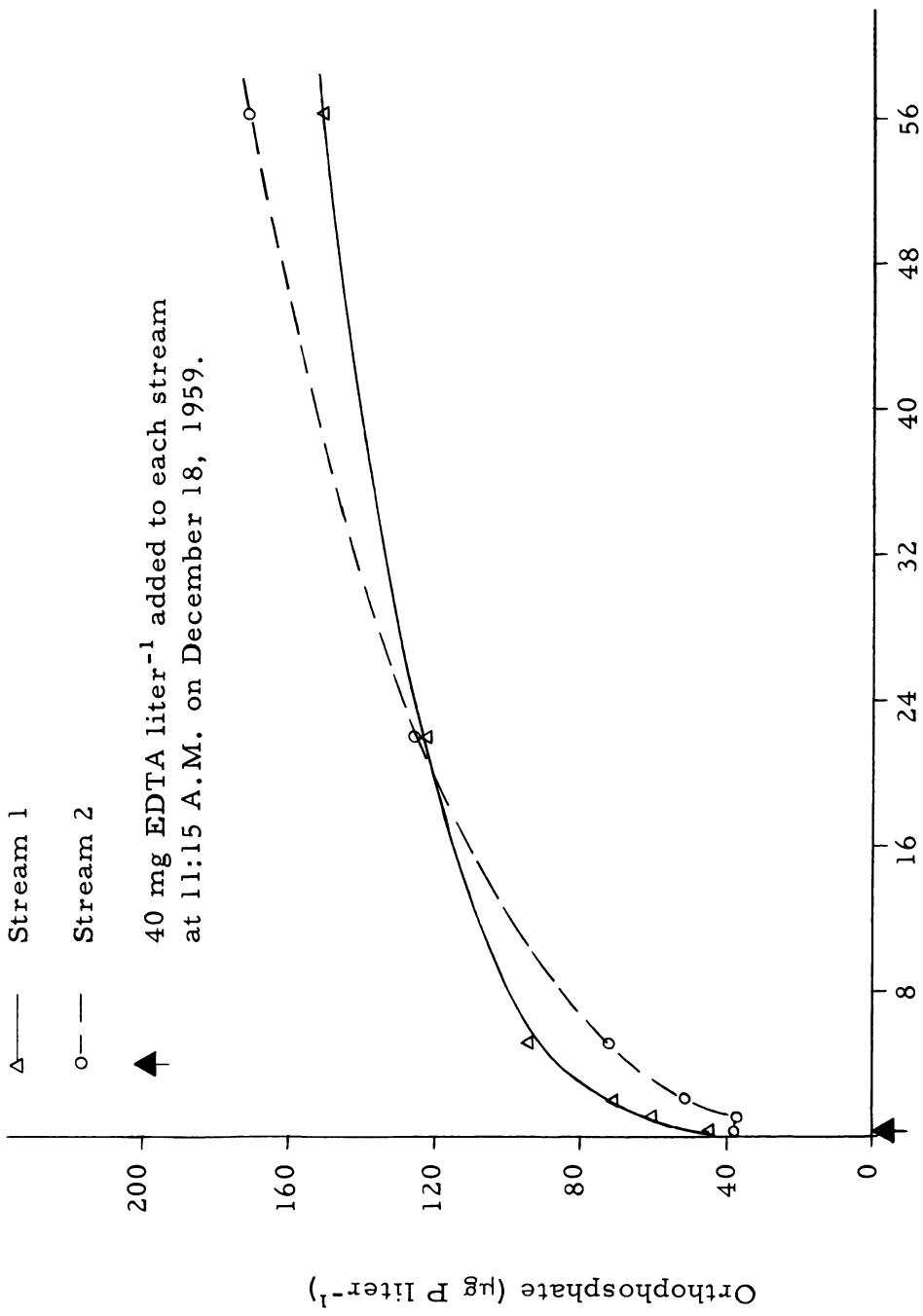

Stream 1
Stream 2
40 mg EDTA liter-1 added to each stream at 11:15 A.M. on December 18, 1959.
Orthophosphate (µg P liter-1)
200
160
120
80
40
0
8
16
24
32
40
48
56
Hours

Figure 13

Figure 14. The effect of a chelate on net production in artificial streams as measured by carbon dioxide, oxygen, and substrates.

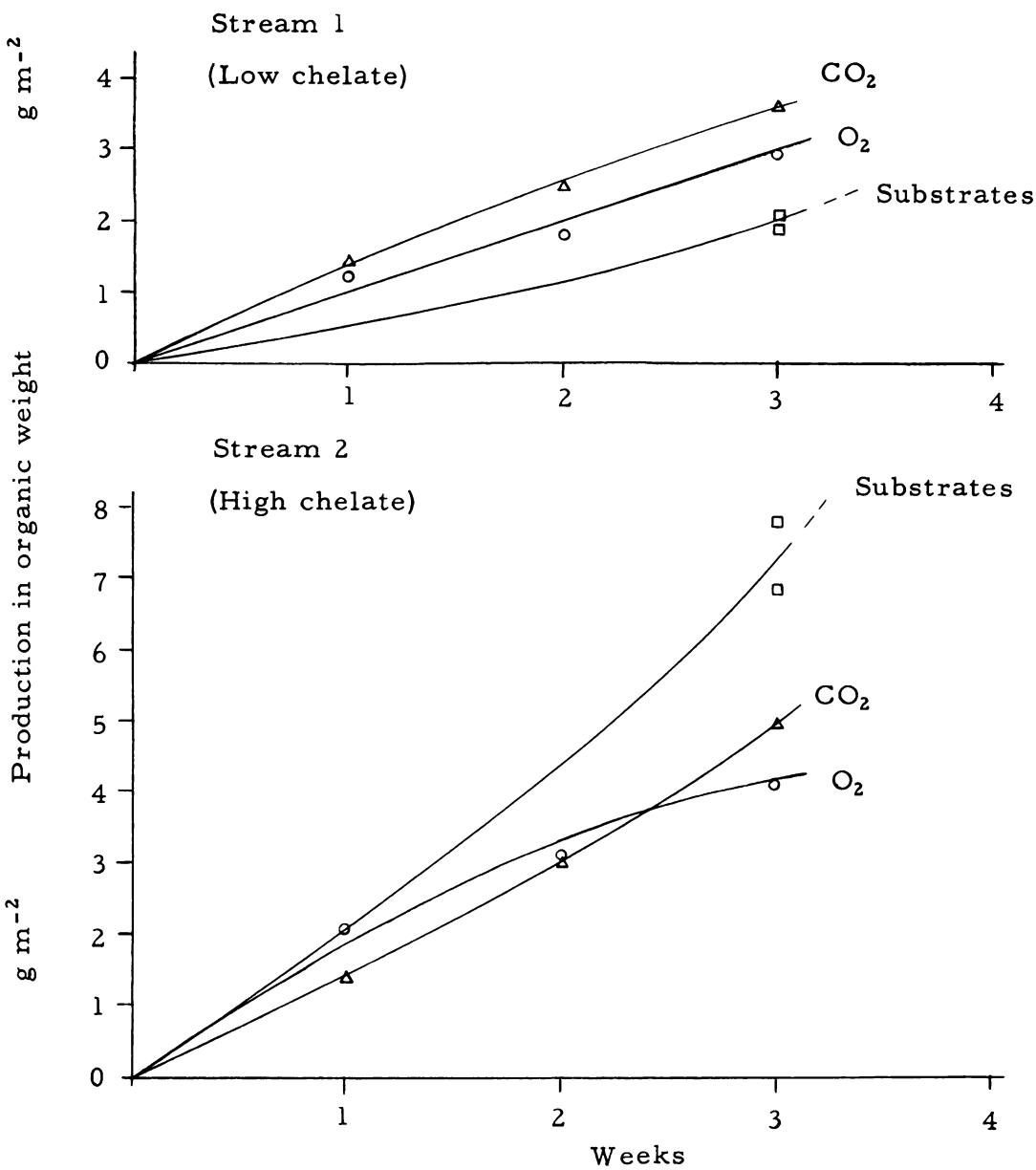

Stream 1
(Low chelate)
CO_2
O_2
Substrates
Stream 2
(High chelate)
Substrates
CO_2
O_2
Production in organic weight
g m^{-2}
g m^{-2}
Weeks
0
1
2
3
4
5
6
7
8

Figure 14

The water chemistry, in Figures 6 and 7, revealed that alkalinity in stream 2 increased while that of stream 1 decreased. The phosphorus concentration in both streams increased, but slightly more in stream 1. Thus the higher productivity in stream 2 cannot be accounted for on the basis of phosphorus release, but is probably due to some unmeasured relationship. It is possible that a limiting metallic ion, less readily chelated, e.g., Mn or Mg, was complexed by the higher chelate concentration in stream 2.

Primary Productivity

The three methods used to measure primary production were the uptake of carbon dioxide, the evolution of oxygen, and biomass collected on artificial substrates. Three methods were used for two reasons: 1) as a combined effort to better indicate the effects of various environmental factors as already shown, and 2) as a means of comparing and evaluating the methods in an effort to determine the method best suited for use in flowing waters.

Growth Rates on Artificial Substrates

The use of artificial substrates to collect periphyton as a measure of primary production has only been considered in recent years. Fortunately most of the recent reports about artificial substrates have contributed important information concerning their use. Newcombe (1950) reported that the percent of organic matter was greater for algae from the undersides of slides because of the silt that settled on the tops. Newcombe (1949) also noted that periphyton on slides was representative of the natural population, but Lund and Talling (1957) theorized that slides would have to favor the pioneer species of the community. Peters (1959) investigated this subject and reported, like Newcombe (1949),

that slides were not selective. Castenholz (1960) also stated slides to be non-selective.

One of the most important and difficult questions yet to be answered is what substrate exposure time gives the most representative sample of periphyton. Most investigators using artificial substrates have selected a time period that allowed a perceptibly good coating of periphyton to accrue on the substrate. This period is usually reported as being between 10 days and four weeks and is practical, but does not answer the question. Two questions arise to be answered before an adequate answer to the first question can be determined. In what part of the population growth pattern is the periphyton on artificial substrates? And does this phase of growth on the artificial substrates represent the over-all condition on the stream bottom where the periphyton is usually in all combinations of the growth pattern?

An attempt to answer these questions was made by studying the accrual of periphyton on substrates in the artificial streams. Substrates were removed every 2 or 3 days up to 34 days. It was thus possible to determine the colonization period and to follow the subsequent rates of growth. Lund and Talling (1957) maintain that the complexity of environments makes attempts to use mathematical models to describe the population growth of algae unprofitable. Control of the environment in the artificial streams and the great predominance of one algal species avoided some of the complexities found in natural ecosystems.

The question involving the pattern of growth on the bottom of the streams was investigated indirectly. The rate of periphyton growth on the substrates was compared to rates measured by the pH-carbon dioxide method. Both methods measure net production; however, the carbon dioxide method measures the net production of the entire community thus affording a comparison of growth on substrates with growth

of the long established population on the stream bottom. This would rarely be feasible in natural systems since the carbon dioxide method would include the changes caused by the metabolism of fauna and higher aquatic plants which were absent in the artificial streams.

The study of growth rate was incorporated with the study of current velocity and results reflect different growth rates for the two streams. The data indicate that growth on substrates follows the sigmoid pattern at least into the logarithmic phase (Figure 15). Inspection of the substrates revealed the first periphyton to become measurable no earlier than three days and this period of time was subtracted from the exposure periods of the shingles for the growth rate study. Thus in Figure 15, day 0 corresponds to day 3 making an initial 3 days of no standing crop which does not appear on the graph. This initial period is required for establishment of the periphyton on the substrate and should not be included in calculations of productivity.

The sigmoid growth curve can be divided into three sections, the initial slope being the lag phase, the steep slope being the logarithmic phase, and the final slope approaching the asymptote being the senescent phase. When the data were plotted on a logarithmic scale the lag phase and logarithmic phase became obvious (Figure 16). The lag phase extended through approximately 12 days in stream 1 and about 13 to 14 days in the slower current of stream 2. The data were converted to productivity values and plotted by riffle and pool in Figures 17 and 18. The data fit the typical growth rate curves and indicate in both riffles and pools that the periphyton on the shingles at 34 days is at or near the maximum rate and further samples would probably have continued at the same rate or would have shown a decline. This decline would be the senescent phase and would continue on the lines of Figure 15 reaching the asymptote in about another 30 days. Generally the periphyton coating on the artificial substrates begins to slough off before it proceeds far

Figure 15. Biomass of periphyton on substrates exposed for various time periods in the artificial streams.

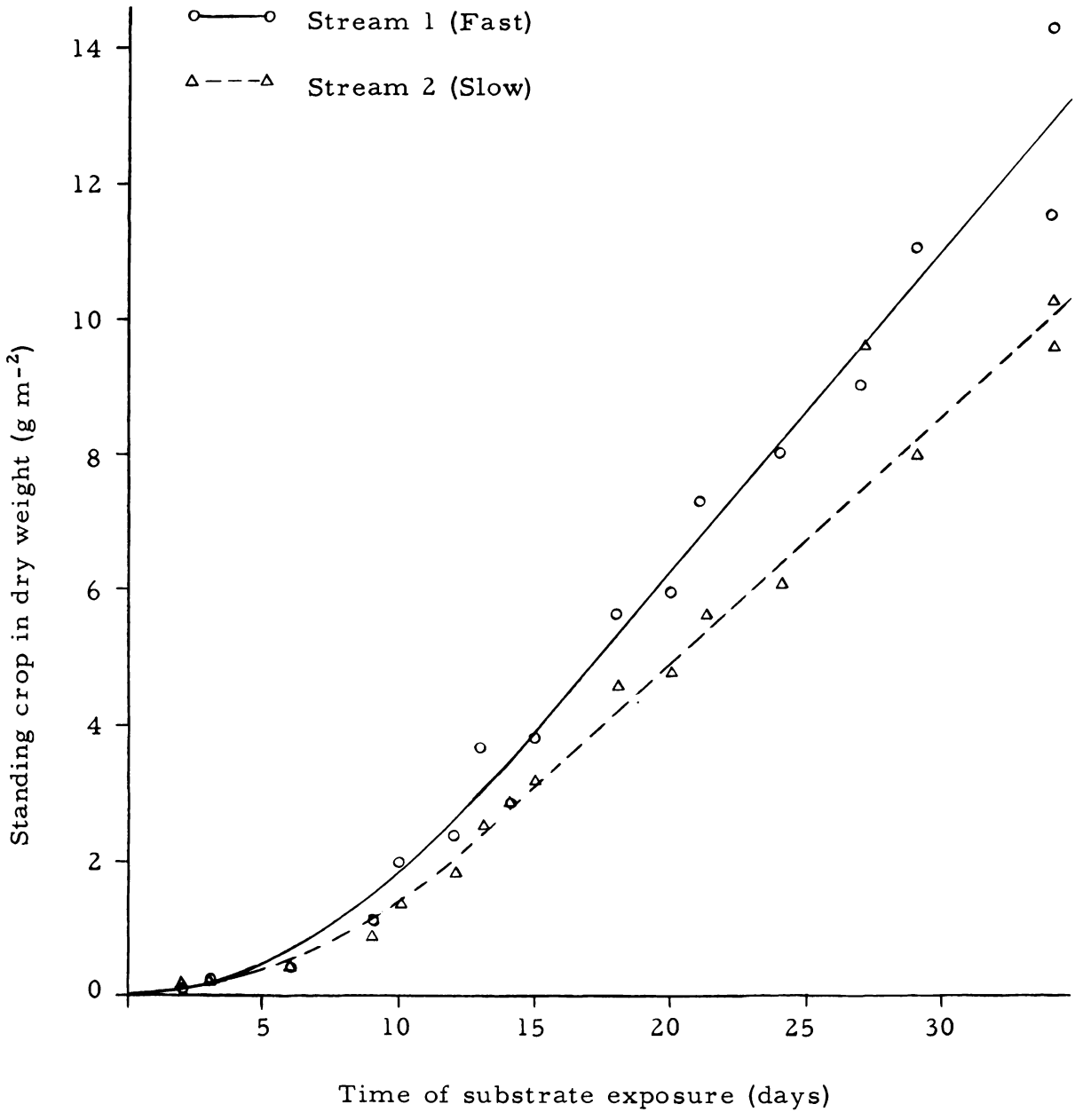

Stream 1 (Fast)
Stream 2 (Slow)
Standing crop in dry weight (g m-2)
0
2
4
6
8
10
12
14
5
10
15
20
25
30
Time of substrate exposure (days)

Figure 15

Figure 16. Growth phases of periphyton on substrates exposed in artificial streams.

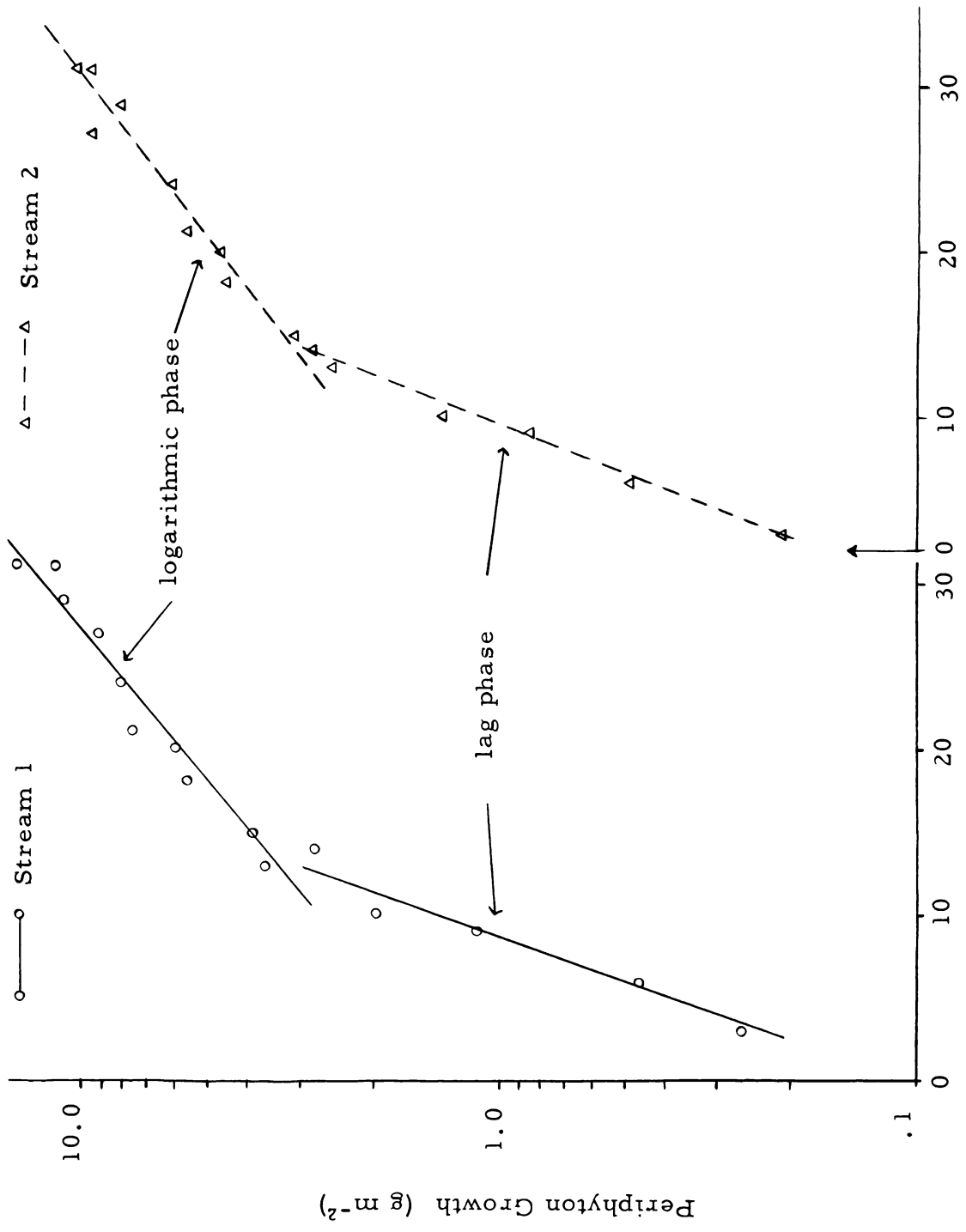

Stream 1
Stream 2
logarithmic phase
lag phase
Periphyton Growth (g m⁻²)
10.0
1.0
.1
0
10
20
30
0
10
20
30
Time of substrate exposure (days)

Figure 16

Figure 17. Rate of periphyton growth on substrates in stream 1 during current velocity study.

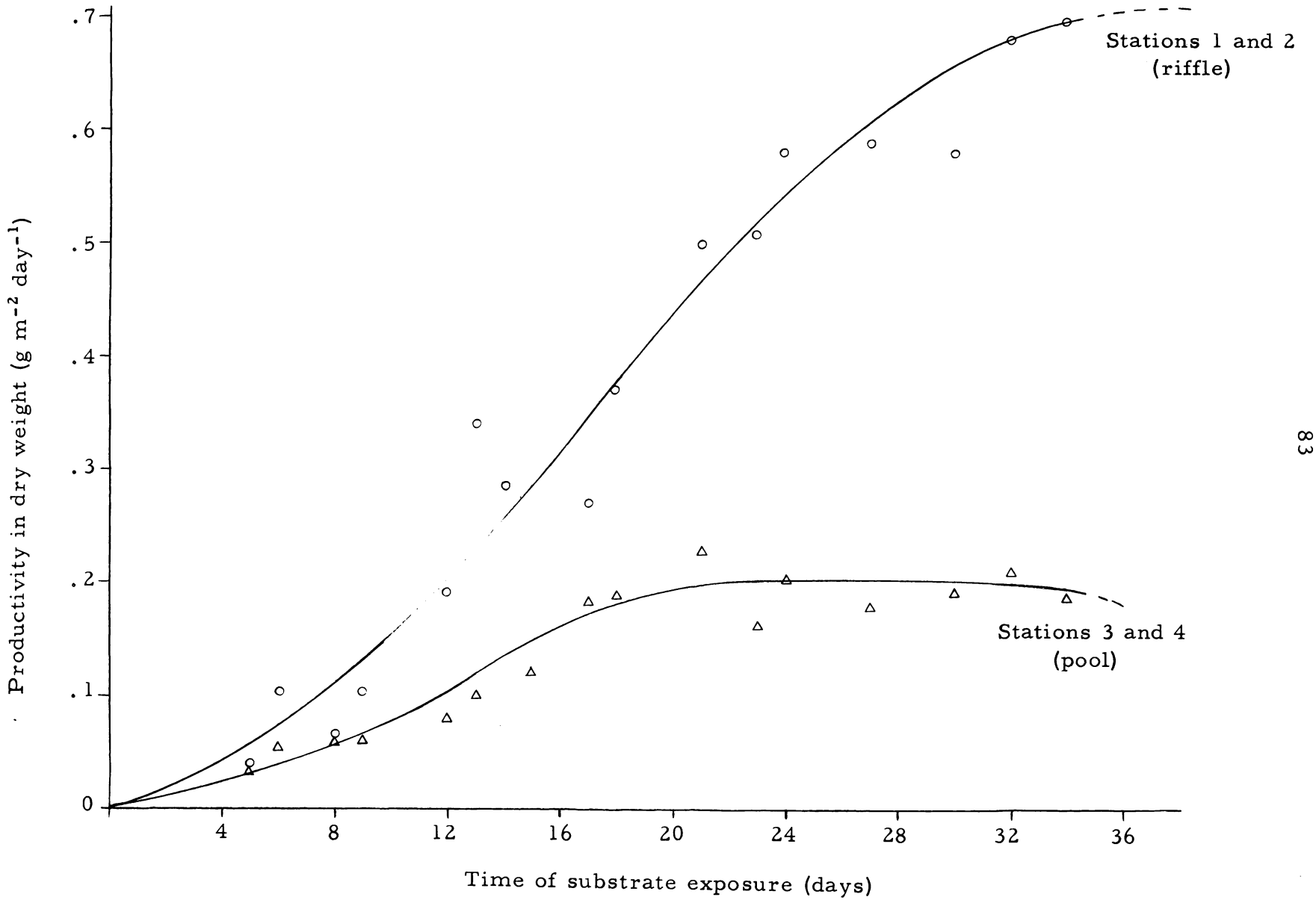

.7
.6
.5
.4
.3
.2
.1
0
Productivity in dry weight (g m^{-2} day^{-1})
4
8
12
16
20
24
28
32
36
Time of substrate exposure (days)
Stations 1 and 2
(riffle)
Stations 3 and 4
(pool)

Figure 17

Figure 18. Rate of periphyton growth on substrates in stream 2 during current velocity study.

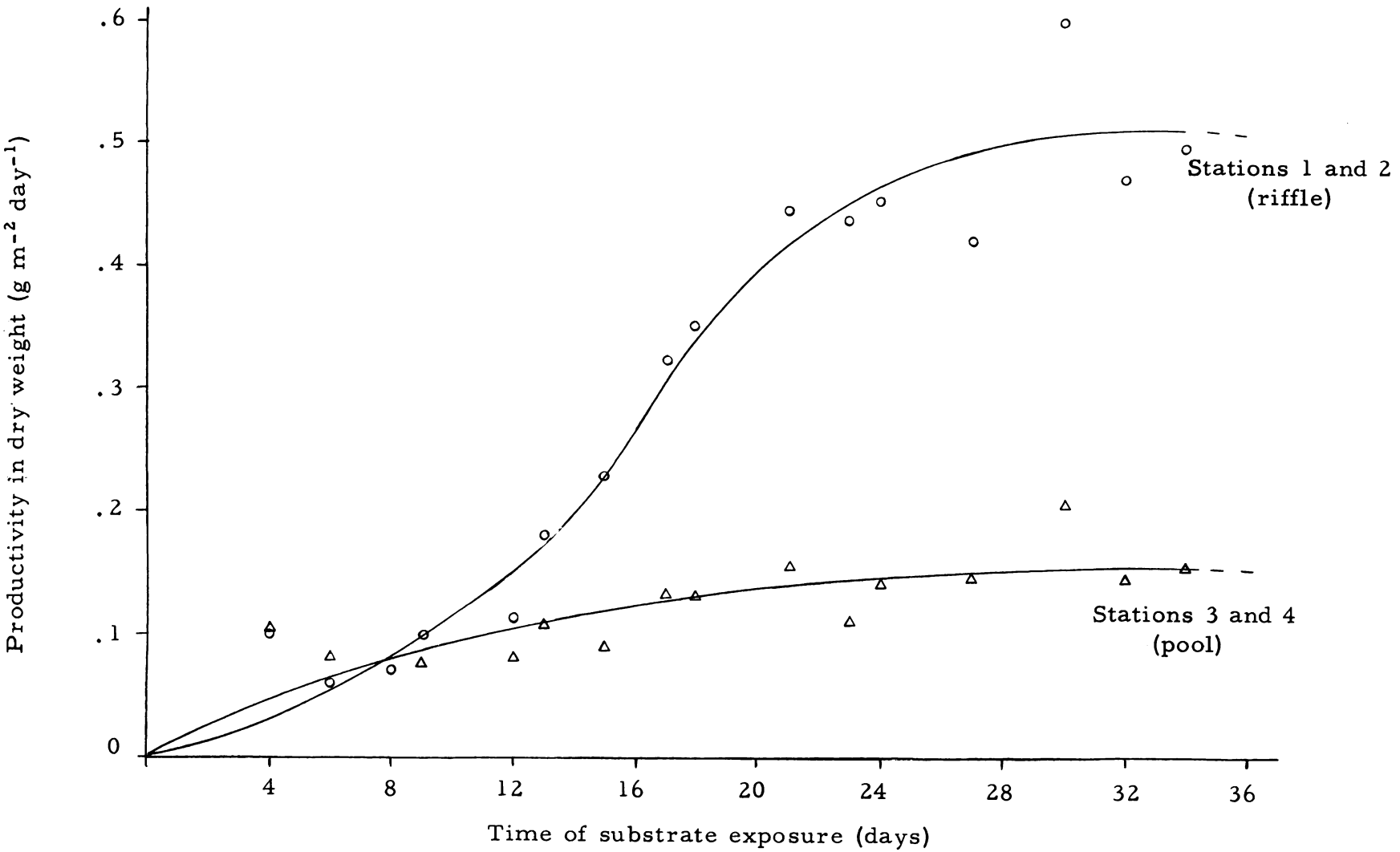

.6
.5
.4
.3
.2
.1
0
Productivity in dry weight (g m-2 day-1)
4
8
12
16
20
24
28
32
36
Time of substrate exposure (days)
Stations 1 and 2
(riffle)
Stations 3 and 4
(pool)

Figure 18

into the senescent phase. When sloughing off of the periphyton is considered as part of the growth pattern then the curve has the J-shape growth form. Odum (1959) describes a population of the J-shaped form as dropping directly back to an initial level when the population density reaches some limiting level before the asymptote. This may well be the situation for periphyton mats, at least of the form as that in the artificial streams, i.e., a blue-green algae with a gelatinous matrix. The limiting factors may be a lack of space and light as the upper layers of periphyton "shade out" the underlying filaments adhering to the substrate and cause the death and subsequent loosening of the algae. Another possibility may be as the mat becomes thicker, gas is unable to diffuse through the mat and bubbles are formed which loosen the periphyton.

The growth form of the algae in the artificial streams was further identified by calculation of the growth constant, k, in the exponential equation

$$P_t = P_0 e^{kt}$$

where P_t = the standing crop at time t,

P_0 = the standing crop at time 0,

t = the time in days,

and e = the base of natural logarithms.

Calculations were based on values taken from Figure 15. During the current velocity study the growth constant for the logarithmic phase of stream 1 was 0.0692 and for stream 2, k = 0.0676. McCombie (1960) studied cultures of green algae and reported a range for k of 0.02 to 0.1 under a temperature range of 5 to 25 C and a light range of 150 to 200 ft-c. Riley (1941) reported k = 0.345 for plankton growth on Georges Bank when productivity was 1 g m^{-2} day^{-1} dry weight. The productivity for the artificial streams was roughly 0.3 g m^{-2} day^{-1} dry weight for the period used to calculate the growth constants.

The turnover rate and turnover time for biomass were also calculated for both streams for the current velocity study. Turnover rate is defined as the fraction of the total amount of algae in the ecosystem which is produced in a given time period (Odum and Odum, 1955). The turnover time is the reciprocal of the turnover rate or the time necessary to produce an amount of algae equal to the standing crop. Standing crop for both streams was estimated to be about 11.0 grams of ash-free dry weight per square meter. The productivity for stream 1 was estimated as 0.367 g m^{-2} day^{-1} ash-free dry weight.

$$\text{Turnover Rate} = \frac{.367 \text{ g m}^{-2} \text{ day}^{-1}}{11 \text{ g m}^{-2}} = 0.0333 \text{ day}^{-1} \ (3.33\% \text{ day}^{-1})$$

$$\text{Turnover Time} = \frac{1}{.0333} = 29.97 \text{ days or } 12.2 \text{ turnovers year}^{-1}$$

The same calculations for stream 2 were:

$$\text{Turnover Rate} = \frac{.314}{11} = 0.0285 \text{ day}^{-1} \ (2.85\% \text{ day}^{-1})$$

$$\text{Turnover Time} = \frac{1}{.0285} = 35.09 \text{ days or } 10.4 \text{ turnovers year}^{-1}$$

Comparisons between productivity rates as measured by substrates and by carbon dioxide reveal the rates by substrates to be consistently lower during the studies of effects of environmental factors. A review of Figure 15 shows that the rate of production (standing crop divided by exposure time) increases with increasing exposure time. Seemingly then, the best estimates of productivity are made from data from substrates with the longest exposure times. This is logical since the periphyton on the substrates exposed longest more nearly parallel the algae on the stream bottom. Moreover, the periphyton growth rate on the substrates most similar to that on the stream bottom is that growth rate occurring toward the end of the exposure period. Therefore if the productivity is calculated for the growth occurring during the last three

days of a 30 day substrate exposure period, the estimate should approach that based on the periphyton of the stream, provided the algae of the two sites are in comparable phases of the growth pattern. During the current velocity study, substrate pairs were removed at frequent intervals thus allowing subtraction of the growth of one period from that of a longer period.

Measurements for carbon dioxide at 28 days during the current velocity study yielded net productivity values of 0.403 g m^{-2} day^{-1} organic weight for stream 1 and 0.359 g m^{-2} day^{-1} for stream 2 (Figure 12). Carbon dioxide values are low since respiration in the reservoirs is included. Substrate data from Figure 15 for a 28 day exposure time yield productivity values in g m^{-2} day^{-1} organic weight for streams 1 and 2 of 0.327 and 0.250 respectively. When the standing crop at day 25 is subtracted from the standing crop at day 28 and productivity calculated for the intervening 3 days, the results for streams 1 and 2 are increased to 0.424 and 0.326 g m^{-2} day^{-1} respectively thus becoming closer to the estimates by the carbon dioxide method.

The collection of periphyton on artificial substrates appears to provide a good means of estimating primary productivity when the exposure period is long enough to allow the collected periphyton to approach a growth phase similar to that of the periphyton on the stream bottom. The most valid estimates are obtained when calculations are based on growth accrued during the last few days of the exposure period. Productivity based upon substrate data does not include any production by higher aquatic plants.

Diurnal Gas Changes

The comparison of productivity values estimated from oxygen and carbon dioxide data was preceded by a number of preliminary calculations and assumptions. Before oxygen and carbon dioxide methods

can be compared the data must be converted into some common unit. Since measurements also included biomass it was decided to convert all values to ash-free dry weight. Conversion of gas measurements to organic weight requires a knowledge of or assumptions concerning the photosynthetic product. Many investigators have in the past assumed the organic matter produced to be entirely carbohydrates. This assumption may be warranted in view of the other errors involved, but at the same time the fullest use should be made of available information. Myers and Cramer (1947) stated that in growing cultures of Chlorella as much protein was synthesized as carbohydrate. Moyse (1959) also reported that materials other than carbohydrates were synthesized during photosynthesis and noted rapid production of amino acids during low light intensity.

Estimations of the composition of the algae produced in the artificial streams were based on measurements of the nitrogen content of the algae, on the caloric values of the algae, and on reports of the fat content of blue-green algae. The mean organic nitrogen, based on 62 Kjeldahl determinations made throughout the investigation, was calculated as 0.0381 mg N per mg dry weight of algae. This value times the conventional factor of 6.25 indicated the algae to be 24% protein. The blue-green algae are reported to have a low lipid content, 4% of the dry weight in one case (Strickland, 1960). The mean caloric value, based on 31 determinations, was calculated as 4520 cal g^{-1} of dry weight of algae. Using values of 9500, 5700, and 4000 calories for fat, protein, and carbohydrates respectively, the composition of the algae was determined as 76% carbohydrate, 21% protein, and 3% lipid. This composition gives a caloric value of 4522 calories.

Synthesis of protein indicates the photosynthetic quotient (PQ) may be greater than 1.0. Strickland (1960) discusses PQ values and notes that when nitrate is the source of nitrogen for protein synthesis the PQ

may be as high as 1.6. Ryther (1956) gave a PQ of 1.45 when nitrate was the nitrogen source and cited an average PQ of 1.2. Odum (1957a) also noted PQ values from 1.2 to 1.38 when protein synthesis was high. Metabolism of the oxygen from the nitrate molecule produces a high PQ by increasing the oxygen change of the photosynthetic process ($+ \Delta O_2 / - \Delta CO_2$). Nitrate was the usual source of nitrogen in the artificial streams. On one occasion NH_4NO_3 was used; however, the long term source was recycled nitrogen which would be in the form of nitrate since the stream waters were always high in dissolved oxygen. The respiratory quotient (RQ) is the inverse of the PQ and is influenced by the same factors, but during the day photosynthesis was greater than respiration thus it is reasonable to correct gas values with the PQ. A PQ of 1.0 would indicate an uptake of 6 moles of carbon dioxide, production of 1 mole of carbohydrate, and evolution of 6 moles of oxygen. PQ values were estimated for this study on the basis of literature values and measured caloric values; assuming the caloric values to be an indication of the amount of protein synthesis. The highest caloric values indicated only moderate protein synthesis thus a PQ of 1.3, somewhat less than the quoted literature values, was assumed. Intermediate and low caloric values, still above the carbohydrate level, were assumed to be represented by a PQ of 1.2 and 1.1 respectively. These PQ values were used to determine factors to convert oxygen and carbon dioxide measurements to organic weights.

The organic product of photosynthesis was assumed to have a molecular weight of 180. Although this is the same molecular weight as glucose, it was derived on a different basis. Amino acids in general have a molecular weight less than glucose, but fatty acids have a molecular weight higher than glucose. It was assumed that the opposing effects of amino acids and fatty acids would result in little influence on the molecular weight of the carbohydrate component (76%). If 6 moles of

carbon dioxide were used to synthesize 180 g (1 mole) of organic matter, then a PQ of 1.2 would result in the production of 7.2 moles of oxygen. To place the gases on an organic weight basis, the grams of carbon dioxide were multiplied by 0.682 (1 mole/6moles = 180 g/ 264 g = .682) and with a PQ of 1.2 the grams of oxygen were multiplied by a factor of 0.782 (1 mole/7.2 moles = 180 g/230 g = .782). For PQ's of 1.1 and 1.3, the factors for oxygen were 0.852 and 0.720 respectively.

Measurements of net productivity by oxygen, carbon dioxide, and periphyton collected on substrates are given in Table 7. The oxygen values are corrected for respiration and BOD in the filter boxes and reservoirs thus giving a measurement of productivity for the entire system as does the carbon dioxide data. The substrate data represent net production only for the area of the stream under the lights and thus the differences between the substrate estimates and the gas estimates is even greater than shown.

A two-tailed matched pairs test was used to test if results by the oxygen method in Table 7 were different from results by the carbon dioxide method. The test indicated that the methods are not significantly different at the 5% level (P = .60). Frey and Stahl (1958) reported general agreement among measurements of productivity by oxygen, carbon dioxide, and C^{14} methods using the L-D bottle technique. Odum (1957a) also reported similar results between in situ oxygen and carbon dioxide measurements when results were corrected by a photosynthetic quotient.

According to theory the oxygen method and carbon dioxide method should produce the same estimates and this is suggested by the statistical analysis. The differences that do exist between individual estimates in Table 7 may be due to false assumptions and errors inherent in the methods.

Table 7. Comparison of net productivity in g m^{-2} day^{-1} organic weight as measured by the oxygen, carbon dioxide, and substrate methods.

Study	PQ	Stream No.	Oxygen Method		Carbon Dioxide Method		Substrate Method
			O_2	org. wt.	CO_2	org. wt.	org. wt.
Temperature	1.3	1	.109	.078	.227	.155	.088
		2	.077	.055	.246	.168	.189
		1	.182	.131	.176	.120	.044
		2	.128	.092	.292	.199	.180
Photoperiod	1.3	1	.158	.114	.248	.169	.070
		1	.174	.125	.213	.145	
		2	.108	.078	.255	.174	.037
		2	.261	.188	.194	.132	
		1	.238	.171	.406	.277	.053
		2	.263	.189	.313	.213	.046
Light Intensity	1.2	1	.206	.161	.208	.142	.042
		2	.310	.242	.352	.240	.039
		1	.239	.187	.318	.217	.052
		2	.341	.267	.403	.275	.043
Current	1.1	1	.704	.600	.573	.391	.294
	1.2	2	.535	.418	.558	.381	.241
	1.1	1	.854	.728	.591	.403	.317
	1.2	2	.467	.365	.527	.359	.231
Chelate	1.2	1	.222	.174	.306	.209	.098
		2	.380	.297	.289	.198	.373
		1	.108	.084	.226	.154	.090
		2	.197	.154	.340	.232	.326
		1	.205	.160	.234	.160	
		2	.171	.134	.410	.280	

The formation of gas bubbles under the mat of algae occurred daily. Odum (1957a) claimed that gas in bubbles formed among aufwuchs was not all oxygen because the bubbles remained unchanged at night. The bubbles formed in the artificial streams deflated at night and reappeared during the next day. This phenomenon indicated the gas in the bubbles to be primarily oxygen. Although the oxygen of the bubbles would theoretically be replaced by carbon dioxide at night, the carbon dioxide would tend to diffuse more readily into and through the moist periphyton mat because of the high solubility of carbon dioxide. If the gas of the bubbles is mostly oxygen then measurements by the oxygen method would be lower than the carbon dioxide measurements which was the general case, except for the current study, as shown in Table 7.

Diffusion correction is another critical point in estimating productivity by the gas methods. Burr (1941) noted that carbon dioxide diffused into water 25 times as fast as oxygen, but that oxygen was 700 times as concentrated in air resulting in a net oxygen diffusion 28 times that of carbon dioxide. Thus corrections for oxygen are more critical than for carbon dioxide. The gas transfer coefficients, in g O_2 m^{-2} hr^{-1}, assumed in this study were 0.04 for the slow stream velocity, 0.12 for the fast stream velocity, 0.06 for the stream at 20.0 C, and 0.07 for the stream at 25.6 C and for the streams under standard conditions. It is possible that the coefficient for the faster current is in error and may account for the consistently higher oxygen values of the current study (Table 7); however, the coefficient seems logical in view of the temperature and current conditions.

Diffusion correction for carbon dioxide, although neglected in this study, may be important under conditions of high pH and carbonate ion concentration (CO_2 entry) or low pH and high carbonic acid-carbon dioxide concentration (CO_2 escape). Verduin (1956) calculates 26 mmoles m^{-2} day^{-1} as the highest possible entry of carbon dioxide and in some

cases this would be significant. Cowles and Schwitalia (1923) observed increases of 0.1 pH unit where water flowed over falls and rapids and attributed this to loss of excess carbon dioxide by bubbling.

Since results by the two gas methods are in general agreement, the best method for use in flowing water depends upon stream conditions. The oxygen method requires special attention to obtain measurements for calculation of a gas transfer coefficient, correction for bubbles if they occur in any large amount, and determination of a PQ value if results are to be placed on a biomass or energy basis. Additional requirements are given by Odum (1956). The pH-carbon dioxide method requires the formation of conversion graphs from titration data for each estimate, measurements of the alkalinity of the water, and corrections for diffusion under certain conditions. The pH-carbon dioxide method appears to be the simplest method, but it is limited in accuracy to the more poorly buffered water or, more correctly, to waters yielding measurable pH changes.

Phytopigment Concentration

The concentration of extracted phytopigment was not used to estimate productivity since biomass determinations were also made on all of the samples used for the pigment analysis. The data were collected to determine if the phytopigment method could be used instead of the biomass method. Both methods require the collection of periphyton on artificial substrates; however, the time required for constant weight determinations is much longer than the time needed for extraction and colorimetric readings of the phytopigment. If it can be shown that phytopigment concentration has a definite and consistent relationship to the organic weight and that this relationship can be easily determined then pigment extraction would be the better method to use.

Grzenda and Brehmer (1960) and Peters (1959) showed that a linear relationship existed between phytopigment and organic weight for diatoms of the Red Cedar River, Michigan. Peters (op. cit.) also reported that although a common slope could be used, one regression line could not be used for five different diatom communities. Peters suggested that community differences caused a difference in the phytopigment-organic weight relationship.

Several linear regressions were calculated and compared in this study to see if differences existed in the phytopigment-organic weight relationship of the artificial streams and also to compare the predictor equations with those of other authors. The regression lines of organic weight on phytopigment were calculated for streams 1 and 2 for the overall study, for the light intensity study, and for the current study. The formula used for the regression model was

$$\hat{Y} = a + bx$$

where $\hat{Y}$ = the mean organic weight estimate in milligrams,

a = the intercept of the Y axis,

b = the point estimator of the population slope,

and X = the observed phytopigment reading in phytopigment units.

The coefficient of correlation, r, was also calculated for all regression lines.

Calculation of regression equations for the light intensity study revealed the following:

Stream 1	Stream 2
$\hat{Y} = 1.48 + 90.41X$	$\hat{Y} = 1.11 + 94.32X$
Range of X = .018 to .76	Range of X = .02 to .51
n = 10	n = 10
$r = .9795 > r_{.9995} = .872$	$r = .9586 > r_{.9995} = .872$

An analysis of covariance indicated the slopes and intercepts to be not significantly different at the 5% level (slopes: $P = .90$, intercepts: $P = .90$) thus suggesting that one regression line could be used for the two streams. The common regression equation was

$$\hat{Y} = 1.45 + 91.5X$$

for which $r = .973 > r_{.9995} = .679$.

The following equations and values were calculated for the current velocity study.

Stream 1	Stream 2
$\hat{Y} = 0.2 + 84.57X$	$\hat{Y} = -10.11 + 101.59X$
Range of $X = .005$ to 2.16	Range of $X = .02$ to 1.46
$n = 60$	$n = 66$
$r = .967 > r_{.9995} = .414$	$r = .936 > r_{.9995} = .414$

An analysis of covariance for the two equations for the current velocity study indicated that the slopes were significantly different at the .5% level. Thus a common slope was not calculated.

The combined data for all studies for streams 1 and 2 were subjected to the same analysis.

Stream 1	Stream 2
$\hat{Y} = 1.29 + 83.7X$	$\hat{Y} = -4.40 + 90.2X$
Range of $X = .005$ to 2.16	Range of $X = .02$ to 1.46
$n = 89$	$n = 95$
$r = .969 > r_{.9995} = .361$	$r = .908 > r_{.9995} = .361$

The analysis of covariance testing the regression lines of streams 1 and 2 showed no significant difference between the slopes or between the intercepts at the 5% level (slopes: $P = .25$, intercepts: $P = .75$). The use of a common regression line is suggested by these analyses and calculations resulted in the equation

$$\hat{Y} = 1.34 + 86.08X$$

and a coefficient of correlation of

$$r = .946 > r_{.9995} = .324.$$

The slight difference that exists between slopes of the regression lines for the light intensity study may be due to the effect of the light differences. Sargent (1940) reported that Chlorella under high illumination had a lower chlorophyll content than under low illumination. Yentsch and Scagel (1958) also reported higher pigment concentrations at lower light intensities. The slope difference here also indicates a lower pigment concentration under higher light intensity of stream 2; however, this difference in slopes persists even after the light intensity study and may be due to some other effect.

The differences among the regression equations suggests that various factors may influence the phytopigment-organic weight relationship. At the same time the agreement between the equations for streams 1 and 2 based on the combined data of all studies indicates that these factors, when operating or considered together, may counterbalance one another. This may be a modification of the community homeostasis discussed by Odum (1959) where combined environmental effects, rather than combined species, produces more consistent results.

The regression equation given by Grzenda and Brehmer (1960) is $\hat{Y} = -1.72 + 82.37X$ which agrees well with the common regression line for this study ($\hat{Y} = -1.34 + 86.08$). This agreement implies that a consistent phytopigment-organic weight relationship may exist especially when sufficient data from a variety of community and environmental types are considered. If this is true one regression equation could be used to estimate productivity by the phytopigment method with no more error involved than exists in the other methods. Further evidence in the form of regression equations based on studies in other aquatic ecosystems, is needed to substantiate this hypothesis.

Energy Transfer and Efficiencies

The fixation of radiant energy by aquatic plants is one definition of primary production and the use of energy terminology is the basic and most meaningful way to present production data. The energy dynamics of a biological system, like those of physical systems, are defined by the first and second laws of thermodynamics which are: 1) energy can be neither created nor destroyed, and 2) energy changes and transfers are accompanied by an increase in the entropy of the system thus implying a degradation of the energy and efficiencies less than 100%. These laws are the basis for the statement by MacFayden (1948) that matter circulates, but energy passes through a biological system only once. MacFayden (op. cit.) rightly suggested that the terms "yield" and "productivity" should be excluded from the field of energy measurements. The term "energy transfer" is used here to denote the absorption and fixation of radiant energy by algae.

Estimation of the energy transferred by algae requires measurements of the initial radiant energy impinging on the system and the energy content of the final organic product. The initial radiant energy was represented by two values, 1) the incident light at the surface of the water, L_i, and 2) the available light at the surface of the periphyton mat, L_a (Table 5). The energy content of the algae was determined in calories by bomb calorimetry. The mean and standard deviation for all measurements (n = 31) of caloric values were calculated as 4520 ± 260 g cal. The conversion equation then is

$$\text{g cal} = \text{g organic wt.} \times 4520 \pm 260.$$

Caloric measurements were made for duplicate samples and the mean and standard deviation for the most variable and least variable pair of measurements were 4560 ± 85 and 4945 ± 7 g cal respectively. The calories per gram of organic weight used for each study were as follows:

4923 g cal for the temperature study, photoperiod study, and light intensity study; 4320 g cal for stream 1 and 4498 g cal for stream 2 during the current study; and 4338 g cal for stream 1 and 4400 g cal for stream 2 during the chelate study. These caloric values are the means of calorimetric measurements of algae produced during each particular study.

The conversion equation for dry weight to organic weight was also determined. Based on 214 measurements, the mean and standard deviation of the percent organic weight were calculated as 88.8 ± 7.0 thus

$$\text{mg organic weight} = \text{mg dry weight} \times .888 \pm .07.$$

Strickland (1960) estimated a conversion value for green algae of .85 ± .05 while Milner (1953) reported an ash content of 9.35% for blue-green algae and Schuette (1918) reported an ash content of 6.52% also for blue-green algae.

The energy transfer per square meter per day was calculated for streams 1 and 2 for each study period (Table 8). The calculations were made by multiplying the mean organic weight estimated by the carbon dioxide and oxygen methods (Table 7) by the calories per gram listed for the particular study. The amount of energy transferred by the periphyton was similar for all studies except the current study when daily nutrient supplements were added to the streams. This may indicate that some nutrient was a limiting factor in the other studies, but apparently not to the extent that the effects of the study characteristics were suppressed. The nutrient may have become limiting only after other limiting environmental factors were removed thus establishing an upper boundary for energy fixation and also exemplifying the complexity of biological systems. According to Van Oorschot (1955) low nitrogen concentrations reduced the efficiency of algal cultures. This is apparent in Table 8. When the energy source is constant, lower rates of energy fixation result in lower efficiencies.

Table 8. Energy transfer and efficiencies for periphyton in streams 1 and 2.

Study	Stream Number	Energy (g cal m^{-2} day^{-1})				Efficiencies (percent)			
		L_i	L_a	P_g	P_n	$\frac{P_g}{L_i}$	$\frac{P_g}{L_a}$	$\frac{P_n}{L_i}$	$\frac{P_n}{L_a}$
Temperature	1	68,200	48,240	1451	596	2.1	3.0	0.9	1.2
	2	68,200	48,240	1735	715	2.5	3.6	1.0	1.5
Photoperiod	1	68,200	48,240	2222	691	3.2	4.6	1.0	1.4
	2	68,200	48,240	2006	650	2.9	4.2	1.0	1.3
Light Intensity	1	63,000	44,200	2504	870	4.0	5.7	1.4	2.0
	2	136,800	95,000	3380	1260	2.5	3.6	0.9	1.3
Current	1	68,200	48,240	6026	1994	8.8	12.5	2.9	4.1
	2	68,200	48,240	4356	1485	6.4	9.0	2.2	3.1
Chelate	1	68,200	48,240	1636	612	2.4	3.4	0.9	1.3
	2	68,200	48,240	2236	1096	3.3	4.6	1.6	2.3
$\bar{X}$	1	67,160	47,432	2768	953	4.1	5.8	1.4	2.0
	2	81,920	57,592	2743	1041	3.3	4.8	1.3	1.8
Grand Mean		74,540	52,512	2756	983	3.7	5.3	1.3	1.9

L_i = incident light energy, L_a = available light energy,

P_g = gross energy transfer, P_n = net energy transfer,

P_g/L_i = } Tropic level energy intake efficiency or Lindeman's (1942)
P_g/L_a = } efficiency or photosynthetic efficiency

P_n/L_i = } Production efficiency
P_n/L_a = }

The efficiencies of energy transfer often reveal information not demonstrated by the energy transfer rates. Also comparisons with other ecosystems, where the radiant energy is different, are best made using efficiencies. The efficiencies calculated for Table 8 are based on those established in the literature. The progressive efficiency for trophic levels, λ_n/λ_{n-1}, defined by Lindeman (1942) is represented for primary producers by the ratio of gross energy transfer to incident or available radiant energy (P_g/L_i or P_g/L_a respectively) (Odum, 1959). The efficiencies given by the ratio of net energy transfer to incident or available radiant energy (P_n/L_i or P_n/L_a respectively) were used by Juday (1940) and are represented by Odum (op. cit.) as P_t/P_{t-1} (the trophic level production efficiency) where P is production of biomass and t is a trophic level; t-1 in the case of primary producers representing the source of radiant energy. The efficiencies considered here to be most representative of the ability of algae to transfer energy are those based on the available radiant energy (P_g/L_a and P_n/L_a). Efficiencies based on incident radiant energy include the capacity of the physical system to transmit energy. The ability of the physical system of the artificial streams to transmit energy can be represented by the ratio L_a/L_i and using the grand mean values of Table 8, this is 70.4%.

Calculations for the light intensity study revealed an increase in energy transfer and a decrease in efficiency with an increase in light intensity. Van Oorschot (1955) has reported lower efficiencies in algal cultures when the light intensity was increased and Bonner (1962) has theorized a decrease in efficiency with an increase in light when all other conditions are optimum saying that this phenomenon is a function of light intensity, quantum efficiency, and the light saturation level of the chloroplasts. This phenomenon in the artificial streams may be a function of light relationships as discussed by Bonner (op. cit.) or it may be due to a nutrient limitation on the upper level of energy fixation thus causing the lower efficiency.

An energy flow diagram (Figure 19) was drawn to scale using the grand mean values of Table 8. It is readily apparent that a very small amount of the incident energy is fixed as biomass. The energy unavailable to the periphyton ($L_i - L_a$) is lost in the water phase by reflection, scattering, and absorption. Some available energy is also lost ($L_a - P_g$) by the algae at the bottom of the streams by reflection, transmission, and processes of degradation. Further energy degradation by the algae is through respiration ($P_g - P_n$).

Comparisons are made in Table 9 of the energy fixation rates, as primary productivity, and the efficiencies of the artificial streams with those of other ecosystems. When comparing productivity rates the artificial streams are as low or lower than most of the natural ecosystems. This is because the radiant energy of the artificial streams was only about 1% of the average daylight intensity. Comparisons of efficiencies reveal those of the artificial streams to be higher than all natural ecosystems except those for Silver Springs and other Florida springs for which the values are similar. Efficiencies reported for algal cultures are similar to those of the artificial streams. Kok (1952) reported a mean efficiency, presumed to be P_g/L_a, of 20% when all conditions were optimum and the cultures were dense enough to use all of the available light. Kok's (op. cit.) efficiency agrees exactly with Bonner's (1962) theory that the highest possible efficiency is 20%.

One explanation of the high efficiencies obtained for the artificial streams is based on the light quality of the ecosystem. The predominant wavelengths of the incandescent lights are in the red range. Figure 20 illustrates a comparison of the daylight and incandescent spectra with the absorbancy spectrum of the phytopigment extract. It can be seen that the relative amount of light received in the red range is much greater from the incandescent bulbs than from daylight. The absorbancy of the phytopigment extract is somewhat misleading when the large peak at

Figure 19. Energy flow in the artificial streams based on the mean values of both streams in gram-calories meter^{-2} day^{-1}.

L_i = incident radiant energy
L_a = available radiant energy
$L_i - L_a$ = energy lost in water phase
$L_a - P_g$ = available energy not used in photosynthesis
$L_i - P_g$ = incident energy not used in photosynthesis
$P_g - P_n$ = energy degraded by respiration of algae
$L_i - P_n$ = total energy degradation
P_g = gross energy transfer
P_n = net energy transfer

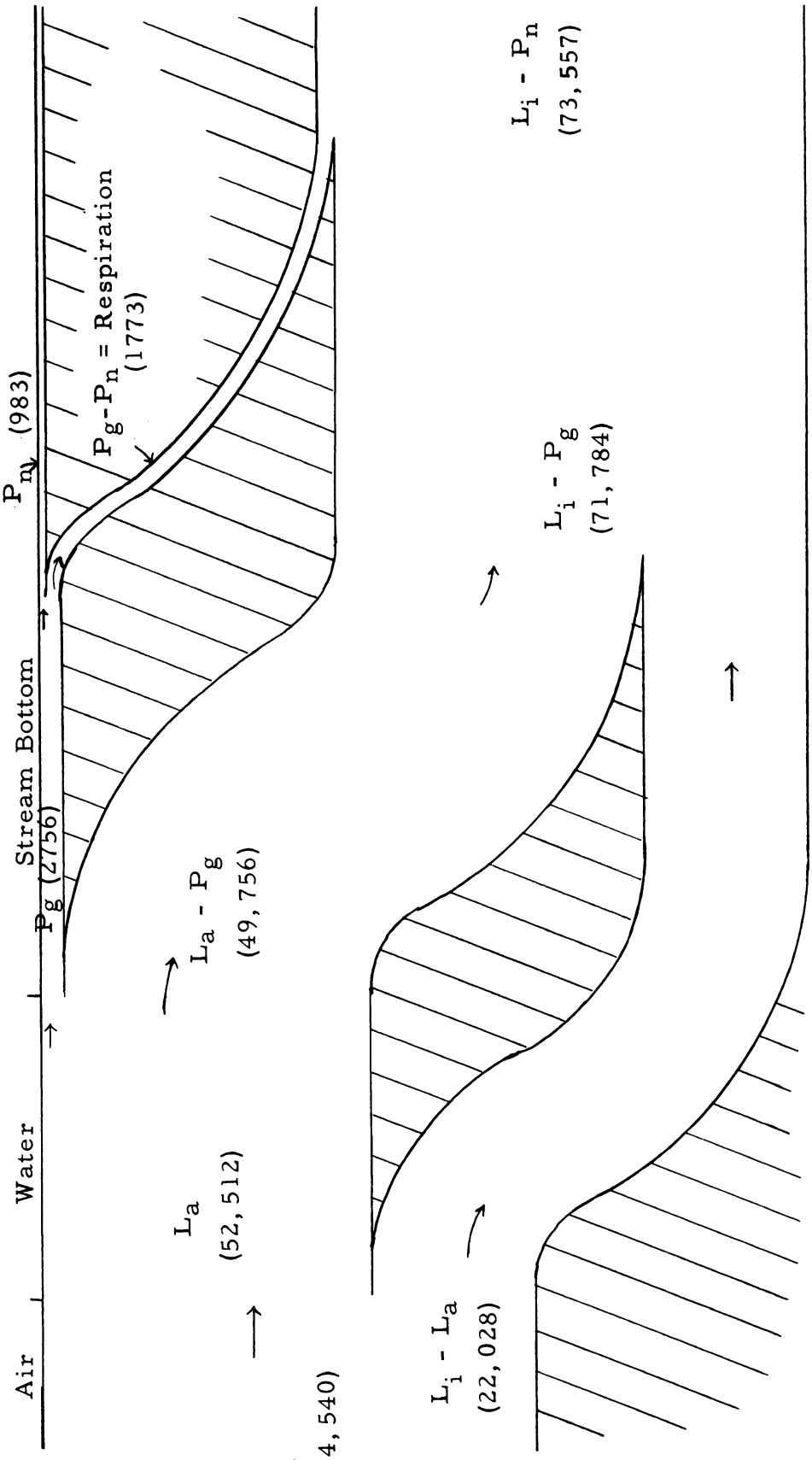

Air
Water
Stream Bottom
P_g (2756)
P_n (983)
$P_g - P_n$ = Respiration
(1773)
L_i
(74, 540)
L_a
(52, 512)
$L_a - P_g$
(49, 756)
$L_i - L_a$
(22, 028)
$L_i - P_g$
(71, 784)
$L_i - P_n$
(73, 557)

Figure 19

Table 9. Comparisons of primary productivity and efficiencies for various ecosystems.

Ecosystem	Net Productivity: g $m^{-2}day^{-1}$ organic wt.	Net Productivity: g cal $cm^{-2}day^{-1}$	Efficiencies (percent): $\frac{P_g}{L_i}$	$\frac{P_g}{L_a}$	$\frac{P_n}{L_i}$	$\frac{P_n}{L_a}$
Artificial[1]	0.10	0.06	8.8	12.5	2.9	4.1
streams	0.46	0.20	2.1	3.0	0.9	1.2
Red Cedar River,[2] Michigan	0.56				0.07	
Cedar Bog Lake[3]		0.19	0.1		0.06	
Minnesota Pond[4]		0.08			0.02	
Silver Springs[5]	7.4	2.42		5.3	0.52	
Root Springs[6]		1.79	0.27			
Lake Mendota[7]					0.27	0.35
Algal culture[8]	1 to 13				1 to 5	
Florida Springs[9]				4.0		
Grand Coulee,[10] Washington	.05 to .8					

[1]Values are the maximum and minimum; [2]Grzenda (1960); [3]Lindeman (1942); [4]Dineen (1953); [5]Odum (1957a); [6]Teal (1957); [7]Juday (1940); [8]Van Oorschot (1955); [9]Odum (1957b), and [10]Castenholz (1960).

L_i = incident light energy, L_a = available light energy,

P_g = gross energy transfer, P_n = net energy transfer,

P_g/L_i = } Tropic level energy intake efficiency or Lindeman's (1942)
P_g/L_a = } efficiency or photosynthetic efficiency

P_n/L_i = } Production efficiency
P_n/L_a = }

Figure 20. Comparison of the daylight and incandescent spectra with the wavelengths absorbed by phytopigment extracts.

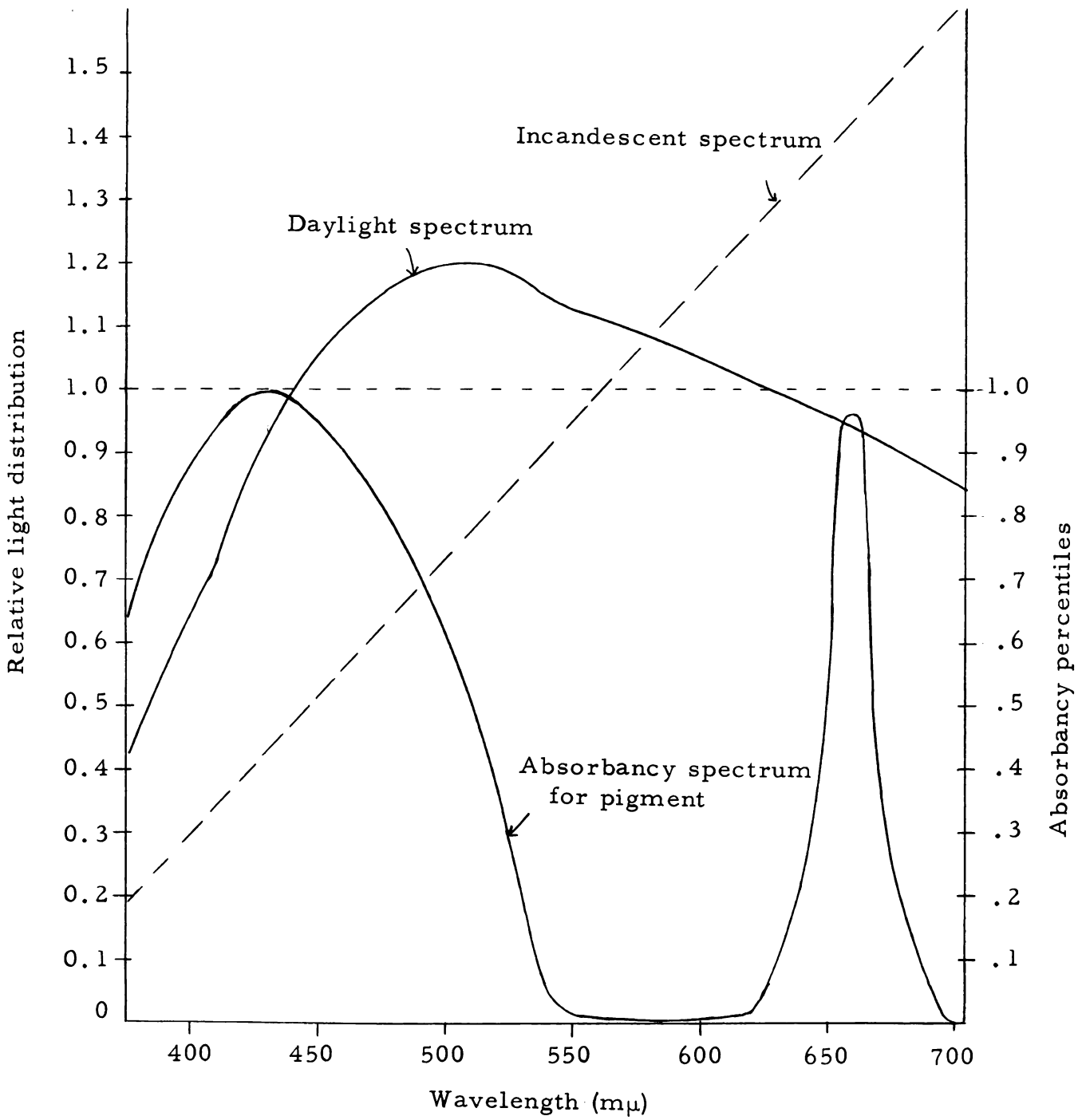

Incandescent spectrum
Daylight spectrum
Absorbancy spectrum
for pigment
Relative light distribution
Absorbancy percentiles
Wavelength (mμ)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
.9
.8
.7
.6
.5
.4
.3
.2
.1
400
450
500
550
600
650
700

Figure 20

425 mμ is noted. According to Noddack and Eichhoff (1939) the maximum absorbancy for living cells of Chroococcus, a blue-green alga, is at wavelengths of 683 mμ (chlorophyll a) and 625 mμ (phycocyanin). Plant physiologists and biochemists have accepted the theory that plants absorb energy in terms of light quanta. It is also known that the energy of a quantum of light decreases as the wavelength of the light increases. A certain number of quanta, regardless of the wavelength, are required to reduce a mole of carbon dioxide in photosynthesis. Thus if the quanta of light are of long wavelengths the efficiency is greater because less energy is required. Kok (1952) reported greater growth in Chlorella cultures with monochromatic light of 650 mμ than with light at 440 mμ. Klugh (1930) also noted the greatest growth of algae under red light when compared to green and blue light. The maximum photosynthetic efficiency for a diatom was given at a wavelength of 655 mμ by Jenkin (1937).

The high efficiencies are also partly explained by considering the general increase in efficiencies when light intensities are decreased (Van Oorschot, 1955, Bonner, 1962).

Nutrient Observations

Nutrient effects were not investigated as a particular phase of the study, but inorganic and organic nitrogen and phosphorus were monitored in an effort to keep the factors influencing productivity limited to the characteristics under study. Water samples were collected weekly for phosphorus and nitrogen analyses and the data for the overall study were compared by streams. If the nutrient levels of the two streams are shown to be similar then further strength is given to conclusions made from the studies of environmental characteristics. Nutrients shown in Figure 7 indicate a general similarity between the two streams.

The nutrient concentrations for the two streams were also subjected to a two-tailed matched pairs test. The statistical analyses showed that the phosphorus concentrations of the two streams were not significantly different at the 5% level ($P = .60$), but the nitrogen concentrations were different at the 1% level of significance ($t = 3.618 > t_{.995} = 2.70$, $df = 43$).

Although the matched pairs test indicated a difference in nitrogen, the difference may not have been critical. The lower concentrations may not have been limiting. Further interpretation of the nutrient concentrations was made after additional analyses of the data. The nitrogen and phosphorus content of the organic weights of the algae were also compared by streams. Since the samples were not taken simultaneously, two-tailed "t" tests were used to compare the means of the organic nitrogen and phosphorus contents of the algae of the two streams. The results of the "t" tests indicated that the phosphorus content and nitrogen content of the algae in stream 1 was not significantly different from those of stream 2 at the 5% level (phosphorus: $P = .60$, nitrogen: $P = .30$). Apparently the difference in the available nitrogen of the two streams did not greatly influence the organic composition of the algae.

A comparison of the two streams in energy transfer rates in Table 8 and nitrogen concentrations in Figure 7 reveals that high nitrogen and high energy transfer coincide in the studies of temperature and light intensity, but are opposite for the two streams in the studies of photoperiod and current velocity. It is assumed then that inorganic nitrogen did not influence algal growth in one stream more than in the other stream.

The biotic utilization of nutrients was investigated using a method described by Grzenda (1960) for a study of the Red Cedar River. The rates of phosphorus and nitrogen fixation were found to increase at similar linear rates with the increase of growth rate (Figure 21). The values for Figure 21 were based on analyses of periphyton collected on

Figure 21. Relationship between nitrogen and phosphorus fixation by periphyton and periphyton growth.

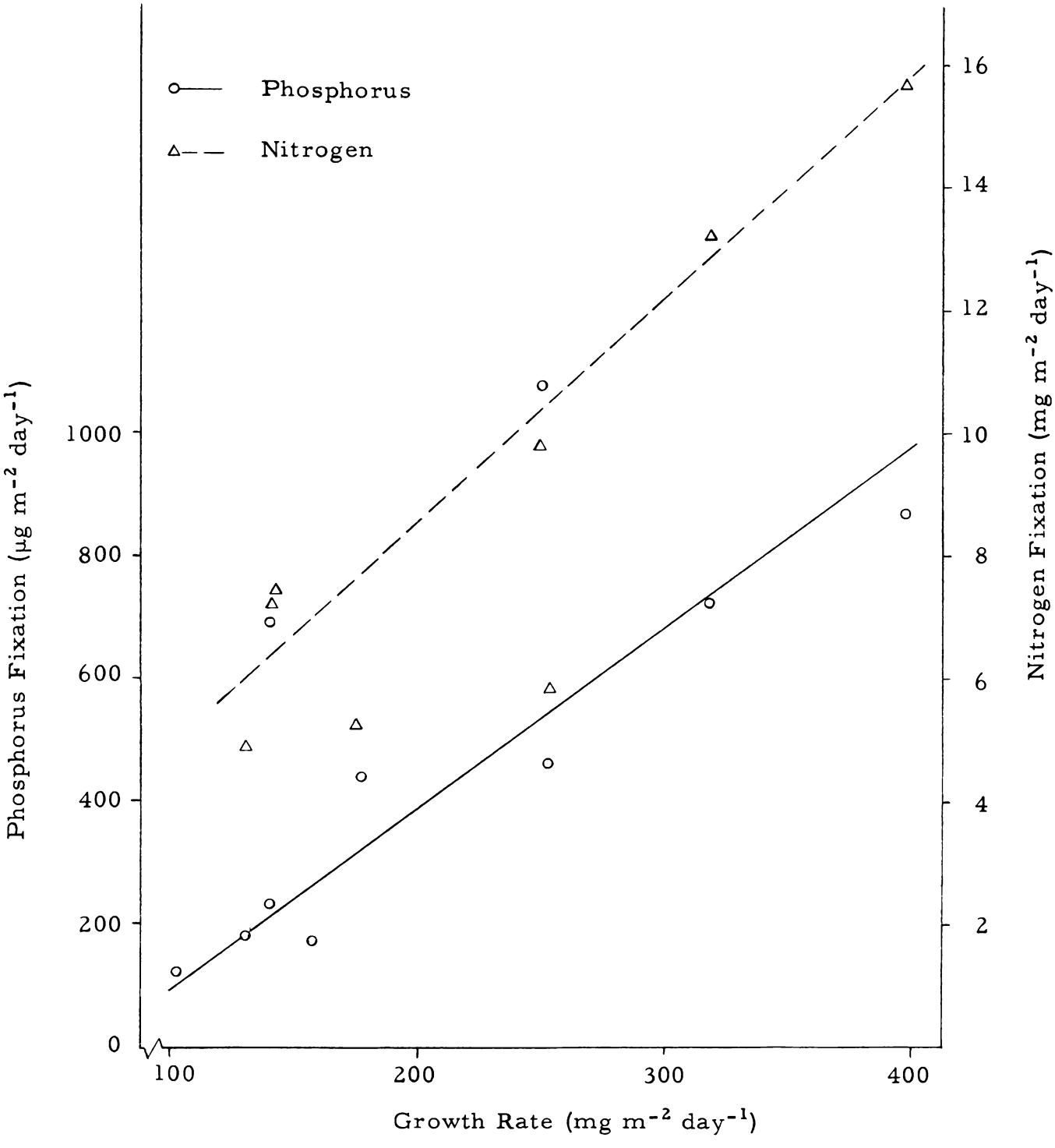

Phosphorus
Nitrogen
Phosphorus Fixation (µg m-2 day-1)
Nitrogen Fixation (mg m-2 day-1)
Growth Rate (mg m-2 day-1)
0
200
400
600
800
1000
2
4
6
8
10
12
14
16
100
200
300
400

Figure 21

artificial substrates assuming the periphyton to be representative of new growth on the stream bottoms. The rate of phosphorus fixation by the blue-green algae in the artificial streams (about 2 μg P mg^{-1} algae) appears to be slightly less than the rate given by Grzenda (op. cit.) for diatoms in the Red Cedar River (about 3.2 μg P mg^{-1} algae), at least in the low growth rate ranges where the two studies are comparable. The higher rate of fixation may be due to the much greater concentration of phosphorus, about 6 fold, in the water of the Red Cedar River or it may be due to a difference in the phosphorus requirements of the algal communities. The rate of nitrogen fixation, although increasing at a rate similar to phosphorus, is much greater per weight of periphyton produced (Figure 21). This reflects the higher nitrogen requirement of the periphyton. The rate of nitrogen fixation was roughly 43 μg N mg^{-1} algae.

The nitrogen and phosphorus content of the periphyton collected on substrates was used to calculate the percent composition of the organic weight (Table 10). Phosphorus calculations were based on the analysis of 113 samples. The mean percent and standard deviation for the phosphorus content of the organic weight was 0.21 ± 0.11. The conversion then from organic phosphorus to organic weight is

$$\text{mg organic wt.} = \text{mg P} \times 476.$$

The capacity of algae to store phosphorus is well-known and is illustrated here by the range of the percent of organic phosphorus from 0.11 to 0.49. Gerloff and Skoog (1954) reported Microcystis aeruginosa to take up phosphorus and nitrogen in proportion to the abundance of the nutrient in the medium and gave organic phosphorus percentages of 0.11 to 0.46. There does not appear to be a relationship between the phosphorus content of the algae and the concentration of phosphorus in the media in Table 10. The lowest phosphorus concentrations (light intensity study)

Table 10. The phosphorus and nitrogen composition and N/P quotients for the organic weight of periphyton from the artificial streams.

Study	Stream Number	Mean nutrient conc. P (μg liter^{-1})	Mean nutrient conc. N (mg liter^{-1})	Organic composition P(%)	Organic composition N(%)	N/P quotient
Temperature	1	20.5	1.90	0.12	--*	--
	2	23.5	3.68	0.11	1.38	12.5
Photoperiod	1	16.4	0.93	0.16	5.17	32.3
	2	16.2	1.26	0.14	3.72	26.6
Light Intensity	1	4.8	0.18	0.26	2.95	11.3
	2	4.0	0.25	0.18	2.28	12.7
Current Velocity	1	13.3	0.28	0.22	4.00	18.2
	2	13.6	0.41	0.23	4.17	18.1
Chelate	1	12.8	0.21	0.49	5.36	10.9
	2	10.0	0.21	0.43	3.94	9.2

*Samples contaminated.

gave intermediate organic phosphorus percentages indicating that phosphorus was not limiting.

The nitrogen content of the periphyton was estimated from the analysis of 62 samples giving a mean percent and standard deviation of 3.29 ± 1.63. The conversion from organic nitrogen to organic weight is

$$\text{mg organic wt.} = \text{mg N} \times 30.4.$$

The nitrogen content of algae is also quite variable as shown by the range in Table 10 of 1.38% to 5.36%. Gerloff and Skoog (1954) reported a range of nitrogen percent of 3.16 to 7.72%, Spoehr and Milner (1949) reported a range of 1.17 to 14.11%, and Ketchum and Redfield (1949), a range of 2.6 to 8.6%. As with the phosphorus data, one of the highest nitrogen concentrations during the studies is paired with the lowest nitrogen content (Table 10) also indicating that nitrogen was probably not a limiting factor.

The ratio of the nitrogen content to the phosphorus content is the N/P quotient and was calculated as 15.9 for the overall study. N/P quotients for the individual studies are given in Table 10. Comparison of the N/P quotients with rates of energy tranfer in Table 8 reveal little relationship; however, the high N/P quotients for the photoperiod study are due to the addition of NH_4NO_3 to the media during that study. The readily available ammonium ion probably caused the high organic nitrogen content. When the N/P quotients of the photoperiod study are disregarded, high N/P quotients appear to be related to high productivities. This is reasonable if one considers rapid growth rates to be accompanied by low phosphorus storage in the algal cells or a high rate of protein synthesis or both.

Since the main supply of nutrients depended upon decomposition of organic matter in the reservoirs and filter boxes, a brief study was undertaken to determine the rate of phosphorus regeneration. Masses of senescent algae were drained and placed in flasks of distilled water or stream water and stored in the dark for a number of days. The orthophosphate concentration of the water was determined upon removal of the flasks from dark storage. The results in Table 11 indicate that the blue-green algae were easily decomposed. The higher rate of phosphorus regeneration occurring in the flasks with stream water may indicate that decomposition was largely by bacteria rather than by

Table 11. Phosphorus regeneration from decomposition of algae placed in 100 ml water and stored in the dark at 25 C.

Water type	Algae in grams wet weight	Incubation in days	Released P^1 in μg	Rate of P^2 released
Distilled	13.433	5.9	44	0.55
Distilled	14.401	7.0	400	4.00
Stream	16.310	2.0	260	8.00
Stream	13,517	11.7	1400	8.85

[1]Released phosphorus as orthophosphate.

[2]Rate in micrograms P per gram of wet algae per day.

autolysis. The rate of phosphorus regeneration occurring in the flask of distilled water incubated for 7.0 days was much higher than in the flask of distilled water incubated for 5.9 days and can be explained by allowing a time period for a bacterial population to build up, since some bacteria must have been present in the stream water adhering to the algae prior to placement of the algae into the flasks. Golterman (1960) studied cycling of elements and reported that 70 to 80% of the organic phosphorus leaves freshly killed algal cells by autolysis in a few days under sterile conditions. Golterman also noted that only the phosphoproteins required breakdown by bacteria. It is difficult to explain the results in Table 11 on the basis of autolysis since the algae were not killed prior to placement in the flasks. If the regeneration did not occur by bacteria or autolysis, it might be explained by metabolic release of phosphorus across cell walls occurring when the algae utilized stored food in the extended period of respiration. This explanation does not account for the difference between distilled and stream water.

Regardless of the explanation it can be assumed that phosphorus regeneration occurred in the dark areas of the artificial streams at a daily rate of approximately 8 μg P g^{-1} algae.

Another brief nutrient study was undertaken to determine the practicality of removing phosphorus from the medium using suspension of kaolin. Suspensions of kaolin were added to each stream until the concentration approached 100 mg $liter^{-1}$. One of the streams was acidified with acetic acid prior to the kaolin addition. The results in Table 12 show that phosphorus was removed from the acidified stream, but not from the unmodified stream. After a period of 3 hours a concentration of 21 μg P $liter^{-1}$ or a total of 5166 μg P had been removed from solution in the acid stream. This is a removal ratio of 21:100, μg P:mg kaolin. The amount of phosphorus removed is small compared to the amount of kaolin added; however, the exposure time was short and the kaolin precipitated rapidly on the stream bottom. If an agitation system were employed the removal efficiency may be increased.

The exact relationship underlying the fixation of phosphorus by kaolinite is not known. Black (1942) has shown that kaolinite has a high affinity for soil phosphorus at pH 4.5 and that this affinity decreases to a low at pH 7.9. Black also demonstrated that a 30 day contact period fixed a much greater amount of phosphorus than a 48-hour period. This indicates that a longer contact period than allowed in the stream studies is necessary for efficient removal of phosphorus. Theories for the action of kaolinite fixation of phosphorus have been given by Black (op. cit) and Hemwell (1957).

Concluding Remarks

The general agreement of results of the environmental studies with theoretical expectations and with similar studies in natural and other laboratory systems indicates several things. The methods used

Table 12. Removal of phosphorus from solution by the addition of 100 mg liter^{-1} kaolin to each artificial stream.

Sampling time	Stream Number	pH	Total alkalinity (mg liter^{-1} $CaCO_3$)	Soluble phosphorus concentration in stream water (μg liter^{-1})
Pre-kaolin	1	5.70	60	74
	2	8.50	103	130
0.5 hr. after kaolin	1	5.84	60	61
	2	8.53	98	124
3 hrs. after kaolin	1	5.86	60	53
	2	8.78	99	133

to measure productivity in flowing water, i.e., pH-carbon dioxide changes, oxygen changes, and periphyton collected on artificial substrates, are sufficiently sensitive to reflect the effects of factors influencing the rate of production. Artificial streams, as used in this study with adequate areas for decomposition and recycling of nutrients and with sufficient time allowed for the systems to become established, are reasonable replicas of natural systems allowing extension and application of the results to streams in general. The magnitudes of the productivities and efficiencies estimated for the artificial streams are also in agreement with those of natural systems indicating that the carbon dioxide and oxygen methods are reasonably accurate as well as sensitive.

SUMMARY

1. Primary productivity in two, artificial, recirculating streams was measured by the pH-carbon dioxide method, the upstream-downstream oxygen method, and by collection of periphyton on artificial substrates.

2. Productivity increased significantly when the environmental characteristics of light intensity, current velocity, and chelate were individually increased.

3. An increase in temperature caused a net productivity increase significant at the 10% level and a Q_{10} value of 1.4 indicating that respiration increased at about the same rate as photosynthesis. The effects of altered photoperiod on net productivity were not significant.

4. Productivity measured by the ash-free dry weight of periphyton collected on artificial substrates was generally lower than estimates by the carbon dioxide and oxygen methods; however, the substrate estimates increased when they were based on the growth rate occurring on the last few days of an extended exposure period. The growth of the periphyton population was described as having a J-shaped form.

5. Estimates of net productivity by the carbon dioxide method and the oxygen method were in general agreement when the oxygen values were corrected by a photosynthetic quotient in the range of 1.1 to 1.3 depending upon the measured caloric values of the periphyton.

6. The relationship between ethanol phytopigment extractions and ash-free dry weights of the periphyton was linear. A common regression equation, indicated by statistical analysis, was calculated with the relationship having a correlation coefficient of .946. The possibility of a consistent phytopigment-organic weight relationship based on community homeostasis is discussed.

7. The flow of energy through the ecosystem was traced from a source of radiant energy from incandescent bulbs to fixation as the chemical energy of organic matter. The periphyton of the artificial streams fixed energy in a range of 0.06 to 0.20 g cal cm^{-2} day^{-1} being somewhat less than the rates of most natural communities. However, the light received by the artificial streams was much less than that of natural communities, being about 1% of the average daylight intensity. Thus efficiencies of productivity were relatively high for the artificial streams and are explained on the basis of light quality and intensity.

8. The efficiency of energy transfer increased when productivity increased in most cases. An increase in productivity caused by a higher light intensity was accompanied by a decrease in the efficiency.

9. Phosphorus concentrations were shown to be similar in the two streams while nitrogen concentrations were different. The effects of the different nitrogen concentrations were negligible as shown by lack of effects on the algal composition and on productivity. The rate of phosphorus and nitrogen utilization increased as productivity increased.

10. The rate of phosphorus regeneration by decomposition of algae was estimated and shown to occur readily in the dark areas of the streams. Phosphorus release from insoluble sources occurred with the addition of a chelate while suspensions of kaolin were used to precipitate phosphorus under acid conditions.

LITERATURE CITED

Abdin, G. 1949. Benthic algal flora of Aswan reservoir (Egypt). Hydrobiologia, 2: 118-133.

Allen, E. J., and E. W. Nelson. 1910. On the artificial culture of marine planktonic organisms. J. Mar. Biol. Assoc. United Kingdom, 8: 421.

Allen, W. E. 1920. A quantitative and statistical study of the plankton of the San Joaquin River and its tributaries in and near Stockton, California, in 1913. Univ. Calif. Publ. Zool., 22: 1-292.

APHA, AWWA, FSIWA. 1955. Standard methods for the examination of water, sewage, and industrial wastes. 10th Ed. Waverly Press Inc., Baltimore Md., 522 pp.

Arnoff, S. 1957. Photosynthesis. Botan. Rev., 23: 65-107.

Atkins, W. R. G. 1926a. A quantitative consideration of some factors concerned in plant growth in water. Pt. I. Some physical factors. J. Conseil Expl. Mer, 1: 99-126.

_____. 1926b. A quantitative consideration of some factors concerned in plant growth in water. Pt. II. Some chemical factors. J. Conseil Expl. Mer, 1: 197-226

Barnes, H. 1957. Nutrient elements. Treatise on marine ecology and paleoecology. Vol. 1. Geol. Soc. Am., Mem. 67: 297-344.

Baumberger, J. P., and R. J. Winzler. 1939. A photometric method for the determination of carbon dioxide. Ind. and Eng. Chem., 11: 371-375.

Beyers, R. J., and H. T. Odum. 1959. The use of carbon dioxide to construct pH curves for the measurement of productivity. Limnol. and Oceanogr., 4: 499-502.

_____. 1960. Differential titration with strong acids or bases vs. CO_2 water for productivity studies. Limnol. and Oceanogr., 5: 228-230.

Birge, E. A., and C. Juday. 1914. A limnological study of the Finger Lakes of New York. Bull. U. S. Bur. Fisheries, No. 32: 525-609.

_____. 1922. The inland lakes of Wisconsin: 1. The plankton, its quantity and chemical composition. Bull. Wisconsin Geol. Nat. Hist. Survey, 64: 1-222.

_____. 1929. Transmission of solar radiation by the waters of inland lakes. Trans. Wisconsin Acad. Sci., 24: 509-580.

_____. 1930. A second report on solar radiation and inland lakes. Trans. Wisconsin Acad. Sci., 25: 285-335.

_____. 1931. A third report on solar radiation and inland lakes. Trans. Wisconsin Acad. Sci., 26: 383-425.

_____. 1932. Solar radiation and inland lakes, fourth report. Trans. Wisconsin Acad. Sci., 27: 523-562.

Black, C. A. 1942. Phosphate fixation by kaolinite and other clays as affected by pH, phosphate concentration, and time of contact. Soil Sci. Soc. Am. Proc., 7: 123-133.

Blum, J. L. 1954. Evidence for a diurnal pulse in stream phytoplankton. Science, 119: 732-734.

_____. 1956. The ecology of river algae. Botan. Rev., 22: 291-341.

_____. 1957. An ecological study of the algae of the Saline River, Michigan. Hydrobiologia, 9: 361-408.

Bonner, J. 1962. The upper limit of crop yield. Science, 137: 11-15.

Burlew, J. S. 1953. Algal culture from laboratory to pilot plant. Carnegie Inst. Wash., Publ. No. 600: 357 pp.

Burr, G. O. 1941. Photosynthesis of algae and other aquatic plants. In A symposium on hydrobiology. Univ. Wisconsin Press, Madison, 405 pp.

Butcher, R. W. 1932. Studies in the ecology of rivers. II. The microflora of rivers with special reference to the algae on the river bed. Ann. Botany, 46: 813-861.

Butcher, R. W. 1946. Studies in the ecology of rivers. VI. The algal growth in certain highly calcareous streams. J. Ecol., 33: 268-283.

Butcher, R. W., F. T. K. Pentelow, and J. W. A. Woodley. 1928. The diurnal variation of the gaseous constituents of river waters. Biochem. J., 21: 945-957.

Castenholz, R. W. 1960. Seasonal changes in the attached algae of freshwater and saline lakes in the lower Grand Coulee, Washington. Limnol. and Oceanogr., 5: 1-28.

Chu, S. P. 1942. The influence of the mineral composition of the medium on the growth of planktonic algae. J. Ecol., 30: 284-325.

Cooke, W. B. 1956. Colonization of artificial bare areas by micro-organisms. Botan. Rev., 22: 613-638.

Cowles, R. P., and A. M. Schwitalia. 1923. The hydrogen ion concentration of a creek, its waterfall, swamp, and pools. Ecology, 4: 402-416.

Dineen, C. F. 1953. An ecological study of a Minnesota pond. Am. Midland Naturlist, 50: 349-376.

Doty, M. S., and M. Oguri. 1959. The carbon-fourteen technique for determining primary plankton productivity. Marine biological applications of radio-isotope research techniques. Suppl. Pubbl. della Sta. Zool. di Napoli, 31: 70-94.

Douglas, B. 1958. The ecology of the attached diatoms and other algae in a small stony stream. J. Ecol., 46: 295-322.

Dvihally, Z. T. 1960. Calculation of underwater distribution of radiant energy as a problem of production biology. Acta Biol., 11: 77-89.

Eddy, S. 1925. Fresh water algal succession. Trans. Am. Microscop. Soc., 44: 138-147.

Ellis, M. M., B. A. Westfall, and M. D. Ellis. 1948. The determination of water quality. U. S. Dept. Int., Fish. Wildl. Ser. Res. Rpt., 9: 122 pp.

Fogg, G. E. 1953. The metabolism of algae. Methuen and Co. Ltd., London, 149 pp.

Fogg, G. E., and D. F. Westlake. 1955. The importance of extracellular products of algae in fresh-waters. Verh. Int. Ver. Limnol., 12: 219-231.

Forbes, S. A. 1887. The lake as a microcosm. Reprint in: Illinois Nat. Hist. Survey Bull., No. 15: 537-550.

Franck, J., and W. E. Loomis, Editors. 1949. Photosynthesis in plants. Iowa State College Press, Ames, 500 pp.

Frey, D. G. 1956. Symposium on primary production: Preface. Limnol. and Oceanogr., 1: 71.

Frey, D. G., and J. B. Stahl. 1958. Measurements of primary production on Southhampton Island in the Canadian Arctic. Limnol. and Oceanogr., 3: 215-221.

Gaarder, T., and H. H, Gran, 1927. Investigations of the production of plankton in the Oslo Fjord. Rapp. et Proc.-Verb., Cons. Expl. Mer, 42: 1-48.

Gardiner, A. C. 1943. Measurement of phytoplankton by the pigment extraction method. J. Mar. Biol. Assoc. United Kingdom, 25: 739-744.

Gerloff, G. C., and F. Skoog. 1954. Cell contents of nitrogen and phosphorus as a measure of their availability for growth of Microcystis aeruginosa. Ecology, 35: 348-353.

Gessner, F., and F. Pannier. 1958. Influence of oxygen tension on respiration of phytoplankton. Limnol. and Oceanogr., 3: 478-480.

Golterman, H. L. 1960. Studies on the cycle of elements in fresh water. Acta Botan. Neerlandica, 9: 1-58.

Grzenda, A. R. 1960. Primary production, energetics, and nutrient utilization in a warm-water stream. Unpub. Doctor's thesis, Mich. State Univ. Lib., 99 pp.

Grzenda, A. R., and M. L. Brehmer. 1960. A quantitative method for the collection and measurement of stream periphyton. Limnol. and Oceanogr., 5: 190-194.

Gumtov, R. B. 1955. An investigation of the periphyton in a riffle of the West Gallatin River, Montana. Trans. Am. Microscop. Soc., 74: 278-292.

Harvey, H. W. 1934. Measurement of phytoplankton population. J. Mar. Biol. Assoc. United Kingdom, 19: 761-773.

Hemwell, J. B. 1957. The fixation of phosphorus by soils. Advances in Agronomy, Academic Press Inc., New York, 9: 95-112.

Hentschel, E. 1916. Biologische Untersuchungen über den tierischen und pflanzlichen Bewuchs im Hamburger Hafen. Mitteil. aus d. Zool. Mus. Hamburg, 33: 1-172.

Hill, R., and C. P. Whittingham. 1955. Photosynthesis. John Wiley and Sons, Inc., New York, 165 pp.

Hoskin, C. M. 1959. Studies of oxygen metabolism of streams of North Carolina. Publ. Inst. Marine Sci., 6: 186-192.

Hutchinson, G. E. 1957. A treatise on limnology. John Wiley and Sons, Inc., Vol. 1: 1015 pp.

Ichimura, S., and Y. Aruga. 1958. Some characteristics of photosynthesis of fresh water phytoplankton. Botan. Mag. (Tokyo), 71: 261-269.

James, H. R., and E. A. Birge. 1938. A laboratory study of the absorption of light by lake waters. Trans. Wisconsin Acad. Sci., 25: 1-154.

Jenkin, P. M. 1937. Oxygen production by the diatom Coscinodiscus excentricus Ehr. in relation to submarine illumination in the English Channel. J. Mar. Biol. Assoc. United Kingdom, 22: 301-343.

Juday, C. 1940. The annual energy budget of an inland lake. Ecology, 21: 438-450.

Juday, C., E. A. Birge, and V. W. Meloche. 1935. The carbon dioxide and hydrogen ion concentration of the lake waters of northeastern Wisconsin. Trans. Wisconsin Acad. Sci., 29: 1-82.

Juday, C., J. M. Blair, and E. F. Wilda. 1943. The photosynthetic activities of the aquatic plants of little John Lake, Vilas County, Wisconsin. Am. Midland Naturalist, 30: 426-446.

Kann, E. 1933. Zur Oekologie des litoralen Algenaufwuchses im Lunzer Untersee. Intern. Rev. ges. Hydrobiol. u. Hydrog., 28: 172-227.

Ketchum, B. H., and A. C. Redfield. 1949. Some physical and chemical characteristics of algae growth in mass culture. J. Cell. and Comp. Physiol., 33: 281-299.

Kingsbury, J. M. 1956. On pigment changes and growth in the blue-green alga, Plectonema nostocorum Bornet ex Gomont. Biol. Bull., 110: 310-319.

Klugh, A. B. 1926. The productivity of lakes. Quart. Rev. Biol., 1: 572-577.

_____. 1927. Light penetration into the Bay of Funday and into Chamcook Lake, N. B. Ecology, 8: 90-93.

_____. 1930. Studies on the photosynthesis of marine algae. I. Photosynthetic rates of Enteromorpha linza, Porphyra umbilicalis, and Delesseria sinuosa in red, green, and blue light. Contrib. to Canad. Biol. and Fisheries, 6: 41-63.

Kofoid, C. A. 1903. The plankton of the Illinois River, 1894-1899, with introductory notes upon the hydrography of the Illinois River and its basin. Pt. I. Quantitative investigations and general results. Bull. Illinois State Lab. Nat. Hist., 6: 95-629.

_____. 1908. The plankton of the Illinois River, 1894-1899, with introductory notes upon the hydrography of the Illinois River and its basin. Pt. II. Constituent organisms and their seasonal distribution. Bull. Illinois State Lab. Nat. Hist., 8:1-361.

Kok, B. 1952. On the efficiency of Chlorella growth. Acta Botan. Neerlandica, 1: 445-467.

Kramer, C. Y. 1956. Extension of multiple range tests to group means with unequal number of replications. Biometrics, 12: 307-310.

Kratz, W. A., and J. Meyer. 1955. Nutrition and growth of several blue-green algae. Am. J. Botany, 42: 282-287.

Kreps, E., and N. Verjbinskaya. 1930. Seasonal changes in the Barents Sea. J. Conseil Exptl. Mer, 5: 327-345.

Krogh, A., and E. Lange with W. Smith. 1930. On the organic matter given off by algae. Biochem. J., 24: 1666-1671.

Lindeman, R. L. 1942. The trophic-dynamic aspect of ecology. Ecology, 23: 399-418.

Lund, J. W. G., and J. F. Talling. 1957. Botanical limnological methods with special reference to the algae. Botan. Rev., 23: 489-583.

Lyman, J. 1961. Changes in pH and total CO_2 in natural waters. Limnol. and Oceanogr., 6: 80-82.

MacFadyen, A. 1948. The meaning of productivity in biological systems. J. Animal Ecol., 17: 75-80.

Manning, W. M., and R. E. Juday. 1941. The chlorophyll content and productivity of some lakes in northeastern Wisconsin. Trans. Wisconsin Acad. Sci., 33: 363-393.

Marsh, C. D. 1899. The plankton of fresh water lakes. Trans. Wisconsin Acad. Sci., 13: 163-187.

Marshall, S. M., and A. P. Orr. 1928. The photosynthesis of diatom cultures in the sea. J. Mar. Biol. Assoc. United Kingdom, 15: 321.

McCombie, A. M. 1960. Actions and interactions of temperature, light intensity, and nutrient concentrations on the growth of the green alga, Chlamydomonas reinhardi Dangeard. J. Fish. Res. Bd. Canada, 17: 871-894.

McConnell, W. J., and W. F. Sigler. 1959. Chlorophyll and productivity in a mountain river. Limnol. and Oceanogr., 4: 335-351.

McCleod, G. C. 1958. Delayed light action spectra of several algae in visible and ultraviolet light. J. Gen. Physiol., 42: 243-250.

_____. 1961. Variation of enhancement of photosynthesis with conditions of algal growth. Science, 133: 192-193.

Meier, F. E. 1932. Lethal action of ultra-violet light on a unicellular green alga. Smithsonian Misc. Coll., 87: No. 10, 11 pp.

Milner, H. W. 1953. The chemical composition of algae. In Algal culture, from laboratory to pilot plant. J. S. Burlew, Ed. Carnegie Inst. Wash., Publ. No. 600: 357 pp.

Moore, B., E. B. R. Prideaux, and G. A. Herdman. 1915. Studies of certain photosynthetic phenomena in sea water. Proc. and Trans. Liverpool Biol. Soc., 29: 233-264.

Moore, B., E. Whitley, and T. A. Webster. 1921. Studies of photosynthesis in marine algae. 1. Fixation of carbon and nitrogen from inorganic sources in sea water. 2. Increase of alkalinity of sea water as a measure of photo-synthesis. Proc. Roy. Soc. London, (B) 92: 51-58.

Moyse, A. 1959. Certain aspects of photosynthesis in relation to metabolism of organic acids and amino acids. Fiziol. Rastenii, 6: 287-298.

Myers, J. 1946. Culture conditions and the development of the photosynthetic mechanism. IV. Influence of light intensity on photosynthetic characteristics of Chlorella. J. Gen. Physiol., 29: 429-440.

Myers, J., and M. L. Cramer. 1947. Reconsideration of the photosynthetic mechanism in Chlorella. Science, 105: 552.

Myers, J., and W. A. Kratz. 1955. Relations between pigment content and photosynthetic characteristics in a blue-green alga. J. Gen. Physiol., 39: 11-22.

Newcombe, C. L. 1949. Attachment materials in relation to water productivity. Trans. Am. Microscop. Soc., 68: 355-361.

_____. 1950. A quantitative study of attached materials in Sodon Lake, Michigan. Ecology, 31: 204-215.

Noddack, W., and H. J. Eichhoff, 1939. Untersuchungen über die Assimilation der Kohlensäure durch die grünen Pflanzen. III. Die Lichtabsorption von Algensuspensionen. Z. Physik. Chem., A 185: 241.

Odum, E. P. 1959. Fundamentals of ecology. (In collaboration with H. T. Odum), 2nd Ed., W. B. Saunders Co. Phila., 546 pp.

Odum, H. T. 1956. Primary production in flowing waters. Limnol. and Oceanogr., 1: 102-117.

_____. 1957a. Trophic structure and productivity of Silver Springs, Florida. Ecol. Monographs, 27: 55-112.

Odum, H. T. 1957b. Primary production measurements in eleven Florida springs and a marine turtle-grass community. Limnol. and Oceanogr., 2: 85-97.

Odum, H. T., and C. M. Hoskin. 1957. Metabolism of a laboratory stream microcosm. Publ. Inst. Marine Sci., 4: 115-133.

Odum, H. T., and E. P. Odum. 1955. Trophic structure and productivity of a windward coral reef community on Eniwetok Atoll. Ecol. Monographs, 25: 291-320.

Olson, T. A. 1932. Some observations on the inter-relationships of sunlight, aquatic plant life, and fishes. Trans. Am. Fish. Soc., 62: 278-289.

Osterhout, W. J. V., and A. R. C. Haas. 1918a. On the dynamics of photosynthesis. J. Gen. Physiol., 1: 1-16.

_____. 1918b. The temperature coefficient of photosynthesis. J. Gen. Physiol., 1: 295-298.

Österlind, S. 1947. Growth of a planktonic green alga at various carbonic acid and hydrogen-ion concentrations. Nature, 159: 199-200.

Parr Instrument Company. 1960. Oxygen bomb calorimetry and combustion methods. Technical Manual No. 130. Parr Instrument Co., Moline, Ill., 56 pp.

Peters, J. C. 1959. An evaluation of the use of artificial substrates for determining primary production in flowing water. Unpubl. Master's thesis, Mich. State Univ., 106 pp.

Philip, C. B. 1927. Diurnal fluctuations in the hydrogen ion activity of a Minnesota lake. Ecology, 8: 73-89.

Pietenpol, W. B. 1918. Selective absorption in the visible spectrum of Wisconsin waters. Trans. Wisconsin Acad. Sci., 19: 562-593.

Platt, E. L. 1916. The population of the "Blanket-Algae" of fresh water pools. Am. Naturalist, 49: 752-762.

Prescott, G. W. 1951. Algae of the western Great Lakes area. Cranbrook Institute of Science, Bull. No. 31: 946 pp.

Prescott, G. W. 1956. A guide to the literature on ecology and life histories of the algae. Botan. Rev., 22: 169-240.

Rabinowitch, E. I. 1945. Photosynthesis and related processes. Interscience, New York, Vol. I: 1-599.

_____. 1951. Photosynthesis and related processes. Interscience, New York, Vol. II, Part I: 600-1208.

_____. 1956. Photosynthesis and related processes. Interscience, New York, Vol. II, Part II: 1209-2088.

Rawstron, R. R., Jr. 1961. A study of primary production on artificial substrates in a riffle and pool area of the Red Cedar River. Unpubl. Master's thesis, Mich. State Univ. Lib., 73 pp.

Richardson, B. 1933. Photo-electric measurements of the penetration of light in sea water, and the results of laboratory photo-electric measurements of the absorption coefficient of sea water. Proc. 5th Pacific Sci. Congr. (Canada), Univ. Toronto Press, 1934, 3: 2083-2084.

Riley, G. A. 1941. Plankton studies. IV. Georges Bank. Bull. Bingham Oceanogr. Coll., Peabody Mus. Nat. Hist. Yale Univ. 7: 1-73.

Rodhe, W. 1948. Environmental requirements of fresh-water plankton algae. Symbolae Botanicae Upsalienses, 10: 1-149.

Ruttner, F. 1930. Das Plankton des Lunzer Untersees, seine Verteilung im Raum und in der Zeit. Intern. Rev. Hydro-biol., 23: 1-287.

_____. 1948. Die veränderungen des Äquivalentleitvermögens als Mass der Karbonatassimilation der Wasserpflanzen. Schweiz. Zeitschr. Hydrol., 11: 72-89.

Ryther, J. H. 1956. The measurement of primary production. Limnol. and Oceanogr., 1: 72-84.

Saito, Y. 1958. An ecological study of Undaria pinnatifida Sur. III. On the effects of light intensity and water temperature upon the rate of photosynthesis. Bull. Japanese Soc. Sci. Fish., 24: 484-486.

Sargent, M. C. 1940. Effect of light intensity on the development of the photosynthetic mechanism. Plant Physiol., 15: 275-290.

Saunders, G. W. 1957. Interrelations of dissolved organic matter and phytoplankton. Botan. Rev., 23: 389-409.

Saunders, J. T. 1926. The hydrogen-ion concentration of natural waters. I. The relation of pH to the pressure of carbon dioxide. British J. Exp. Biol., 4: 46-72.

Schelske, C. L. 1962. Iron, organic matter, and other factors limiting primary productivity in a marl lake. Science, 136: 45-46.

Schomer, A. H., and C. Juday. 1935. Photosynthesis of algae at different depths in some lakes of northeastern Wisconsin. Trans. Wisconsin Acad. Sci., 29: 173-194.

Schuette, H. A. 1918. A biochemical study of the plankton of Lake Mendota. Trans. Wisconsin Acad. Sci., 19: 594-613.

Schutow, A. 1926. Die Assimilation der Wasserpflanzen und die aktuelle Reaktion des Milieus. Planta, 2: 132-151.

Scott, W. 1924. The diurnal oxygen pulse in Eagle (Winona) Lake. Proc. Indiana Acad. Sci., 33: 311-314.

Spoehr, H. N., and H. W. Milner. 1949. The chemical composition of Chlorella: Effect of environmental conditions. Plant Physiol., 24: 120-149.

Steeman-Nielsen, E. 1952. The use of radioactive carbon (C^{14}) for measuring organic production in the sea. J. Conseil Expl. Mer, 18: 117-140.

_____. 1960. Dark fixation of CO_2 and measurements of organic productivity. With remarks on chemosynthesis. Physiol. Planatarum, 13: 348-357.

Stephenson, T. A., A. Zoond, and J. Eyre. 1934. The liberation and utilization of oxygen by the population of rock-pools. J. Exp. Biol., 11: 162-172.

Stokes, R. M. 1960. The effects of limiting concentrations of nitrogen on primary production in an artificial stream. Unpubl. Master's thesis, Mich. State Univ. Lib., 81 pp.

Strickland, J. D. H. 1958. Solar radiation penetrating the ocean. A review of the requirements, data, and methods of measurement, with particular reference to photosynthetic productivity. J. Fish. Res. Bd. Canada, 15: 453-493.

_____. 1960. Measuring the production of marine phytoplankton. Fish. Res. Bd. Canada, Bull. No. 122: 172 pp.

Strøm, K. M. 1928. Production biology of temperate lakes. A synopsis based upon recent literature. Intern. Rev. d. ges. Hydrobiol. u. Hydrog., 19: 329-348.

Talling, J. F. 1955. The relative growth rates of three plankton diatoms in relation to underwater radiation and temperature. Ann. Botany, 19: 329-341.

Teal, J. M. 1957. Community metabolism in a temperate cold spring. Ecol. Monographs, 27: 283-302.

Thienemann, A. 1931. Der Prodktionsbegriff in der Biologie. Arch. f. Hydrobiol., 22: 616-622.

Transeau, E. N. 1926. The accumulation of energy by plants. Ohio J. Sci., 26: 1-10.

Tucker, A. 1949. Pigment extraction as a method of quantitative analysis of phytoplankton. Trans. Am. Microscop. Soc., 68: 21-33.

Van der Paauw, F. 1934. Der Einfluss der Temperatur auf Atmung und Kohlensaure assimilation einiger Grunalgen. Planta, 22: 396-403.

Van Oorschot, J. L. P. 1955. Conversion of light energy in algal culture. Mededel. Landbouwhogesch., Wageningen., 55: 225-276.

Verduin, J. 1951. Photosynthesis in naturally reared aquatic communities. Plant Physiol., 26: 45-49.

_____. 1956. Energy fixation and utilization by natural communities in western Lake Erie. Ecology, 37: 40-50.

_____. 1960. Differential titration with strong acids or bases vs. CO_2 water for productivity studies. Limnol. and Oceanogr., 5: 228.

Verduin, J. 1961. Changes in pH and total CO_2 in natural waters. Limnol. and Oceanogr., 6: 82-83.

Viosca, P., Jr. 1935. Statistics on the productivity of inland waters, the master key to better fish culture. Trans. Am. Fish. Soc., 65: 350-358.

Warburg, O., and G. Krippahl. 1960. Neubestimmung des Quantenbedarfs der Photosynthese mit der Kompensations-methode. Zeitsch. Naturforsch., 15b: 190-197.

Warburg, O., W. Schröder, and H. Gattung. 1956. Züchtung der Chlorella mit flukuierender Lichtintesität. Zeitschr. Naturforsch., 11b: 654-657.

Ward, H. B. 1899. Freshwater investigations during the last five years. Trans. Am. Microscop. Soc., 20: 261-336.

Welch, P. S. 1948. Limnological methods. Blakiston Co., Phila., 381 pp.

_____. 1952. Limnology. 2nd Ed. McGraw Hill Inc., New York, 537 pp.

Whitford, L. A. 1960. Current effects and growth of freshwater algae. Trans. Am. Microscop. Soc., 79: 302-309.

Whitford, L. A., and G. J. Schumacher. 1961. Effect of current on mineral uptake and respiration by a fresh-water alga. Limnol. and Oceanogr., 6: 423-425.

Whitney, R. J. 1942. Diurnal fluctuations of oxygen and pH in two small ponds and a stream. J. Exp. Biol., 19: 92-99.

Whittingham, C. P. 1955. Photosynthesis. Endeavour, 14: 173-180.

Wiebe, A. H. 1930. Investigations on plankton production in fish ponds. Bull. U. S. Bur. Fish., 46: 137-176.

Wolle, F. A. 1887. Freshwater algae of the United States. Comenius Press, Bethlehem, Pa., 364 pp.

Yentsch, C. S., and R. F. Scagel. 1958. A diurnal study of phytoplankton pigments, an in situ study in East Sound, Washington. J. Marine Res., 17: 567-583.

Young, O. W. 1945. A limnological investigation of periphyton in Douglas Lake, Michigan. Trans. Am. Microscop. Soc., 64: 1-120.