PHYTOPLANKTON PRODUCTION IN A CENTRAL TEXAS RESERVOIR

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ABSTRACT

Phytoplankton production and other environmental variables were measured from June to November, 1968 to determine factors affecting production and trophic status of the producer community in a shallow, polymictic, central Texas reservoir. The reservoir was highly productive and eutrophic. Net phytoplankton production estimates, derived from ^{14}C data, averaged 390 mg C m^{-3} day^{-1}, 857 mg C m^{-2} day^{-1}, and 300 g C m^{-2} yr^{-1} for the impoundment. Light extinction by organic and inorganic turbidity limited phytoplankton production by decreasing the photic depth. Nutrient limitation was of minor importance, although greater production occurred near points of nutrient inflow than in other reservoir areas.

Wind-mixing of the reservoir is believed to accelerate its eutrophication by promoting rapid nutrient recirculation, and thus maintaining nutrient availability for primary production. The impoundment is expected to age more rapidly with time as its basin shallows and phytoplankters spend an increasingly larger fraction of their lives in the productive zone.

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INTRODUCTION

Aquatic ecosystems characteristically pass through a series of successional phases toward self-extinction. The ageing, or eutrophication, of water bodies has long been recognized as a natural process (Thienemann 1931; Lindeman 1942) and is a result of the gradual increase of biological production. Since inorganic nutrients are necessary for photosynthetic production of organic matter, an increase in nutrient availability may stimulate biological production and promote the premature ageing of a water body. Such accelerated eutrophication is usually attributable to nutrient enrichment caused by man's activities. Eutrophication leads to the degradation of water quality for agricultural, municipal, industrial, and recreational uses, hence is of rapidly growing interest to those concerned with water resources.

Considerable attention has been focused on the accelerated eutrophication of many natural lakes, but there have been few investigations of nutrient inflow effects on impoundment productivity although reservoirs are major public water supply sources for much of the United States. Placement of dams as barriers to natural water drainage systems inherently subjects impoundments to greater nutrient and sediment inflow rates than other lotic ecosystems. An understanding of environmental factors affecting impoundment primary production will aid in maintaining quality water resources for future use.

This investigation determined enriching effects of inflowing nutrients and influences of other environmental factors on phytoplankton

production in a recent central Texas impoundment. The study site was Waco Reservoir, McLennan County, Texas; a flood-control, water-storage impoundment located in the Bosque River drainage basin. My objectives were to (a) determine the trophic status of the reservoir community, (b) study the influence of tributary inflow on phytoplankton production, (c) examine the effect of experimental nutrient enrichment of reservoir water on primary production, and (d) determine the environmental factors which most affect reservoir primary production. The research tested the hypotheses that plant nutrients flowing into Waco Reservoir are assimilated as rapidly as they become available, and that increased nutrient availability results in greater primary production and accelerated eutrophication.

REVIEW OF THE LITERATURE

The Problem of Nutrient Enrichment And Accelerated Eutrophication

Accelerated eutrophication has been consistently related to increased plant nutrient inflow. Nutrient enrichment stimulates primary production, thereby promotes organic and inorganic material accumulation, and increases eutrophication rates. Since autotrophs form the basic energy source for the remainder of the aquatic food chain, nutrient stimulation of primary production may also have beneficial effects. Many workers have fertilized ponds and small lakes to increase fish production (Swingle and Smith 1939; Hasler and Einsele 1948; Ball 1950; Tanner 1960; Hepher 1962; and Olsen and Olson 1965). Although fertilization of certain waters may produce some desirable side effects, nutrient enrichment eventually results in extensive and rapid degradation of large lakes and reservoirs. Water quality degradation from man-caused nutrient enrichment has been reported in the Yahara River lakes at Madison, Wisconsin (Sawyer, Lackey, and Lenz 1944); in several European lakes (Hasler 1947); in Lake Washington, near Seattle (Edmondson, Anderson, and Peterson 1956); in Lake Erie (Harlow 1966); in Lake Zoar, Connecticut (Benoit and Curry 1961); in Clear Lake, California (Goldman and Wetzel 1963); and in Lake Sebasticook, Maine (Mackenthun, Keup, and Stewart 1968). Goldman and Carter (1965) detected eutrophication in oligotrophic Lake Tahoe from cultral waste inflow.

Nutrients Essential for Primary Production

Certain inorganic and organic substances are essential for the photosynthesis of cellular material. Nutrients utilized in large amounts in primary production are carbon, hydrogen, oxygen, sulfur, potassium, calcium, magnesium, nitrogen, and phosphorus. Nitrogen and phosphorus have received major attention due to their low concentrations in most aquatic systems. The law of the minimum (Liebig 1849) implies that the nutrient available in the smallest quality relative to plant growth requirements limits primary production, if other environmental factors are favorable. Phosphorus and nitrogen are most often in such critical supply (Hutchinson 1957; Sawyer 1968).

Many investigators have studied nitrogen and phosphorus concentrations as related to aquatic primary production. Atkins (1923) attempted to relate algal growth to phosphorus uptake in sea water, and concluded that phosphorus might be a limiting factor to phytoplankton production. Birge and Juday (1922) found nitrogen and phosphorus necessary for primary production in Wisconsin lakes. Tucker (1957) reported that Prescott (unpublished) found positive correlations between phosphorus content and phytoplankton production in Iowa lakes. Ketchum (1939) found that phytoplankton growth in sea water was reduced when phosphorus and nitrogen concentration fell below 0.017 mg PO_4-P l^{-1} and 0.047 NO_3-N l^{-1}. Juday et al. (1928), Juday and Birge (1931), and Tucker (1957) found no evidence that phosphorus limited primary production in Wisconsin lakes. Prescott (1939) reported direct correlations between nitrogen content and quantities of phytoplankton in fresh water. Riley (1940) found no effect of phosphorus or nitrogen on primary production, although concentrations of the nutrients correlated closely with variations in

phytoplankton standing crop in Linsley Pond. Changes in the nitrogen-to-phosphorus ratio may have an important influence on phytoplankton production (Pearsall 1932; Cooper 1937; Hutchinson 1941; Gerloff and Skoog 1957; and Goldman and Carter 1965).

Few macronutrients, other than nitrogen and phosphorus, have been found to limit primary production in aquatic systems. Carbon may be limiting in waters very low in bicarbonate (Ruttner 1963). Fish (1955) found that sulfate limited primary production in Lake Victoria, Africa. Goldman and Wetzel (1963) found that sulfate and nitrate limited algal production under bloom conditions in Clear Lake, California. Pearsall (1932) noted that diatom populations increased when English lakes were richest in nitrate, phosphate, and silica. He further observed that diatom growth was limited at silica concentrations of less than 0.5 mg l^{-1}. Lund (1950, 1954) found positive correlations between fluctuations in available silica and diatom periodicity in Lake Windermere, as did Goldman et al. (1968) in Lake Maggiore. Ryther and Guillard (1959) found that silica, iron, and certain trace metals limited phytoplankton growth in the nutrient-poor Sargasso Sea. Nutrient bioassay methods showed that phosphorus and nitrogen were not limiting, although chemical analysis indicated very low environmental concentrations. Menzel and Ryther (1961) found iron to be the primary limiting factor in the Sargasso Sea with phosphorus and nitrogen becoming limiting only when iron was supplied in excess. Tranter and Newell (1963) reported that iron also limited primary production in the Indian Ocean. Menzel, Hulbert, and Ryther (1963) observed that small additions of iron or aluminum in combination with nitrogen and phosphorus stimulated primary production in the Sargasso Sea. They suggested that some nutrients may have a "catalytic function" in phosphorus and nitrogen utilization.

Many micronutrient trace metals and organic growth factors are essential for photosynthetic production. Micronutrient limitation of natural phytoplankton production was first detected by Goldman (1960a). Nicholas (1963) reviewed nutrient requirements of algae and listed nitrogen, phosphorus, calcium, magnesium, sodium, potassium, sulfur, iron, manganese, cooper, zinc, molybdenum, boron, chlorine, cobalt, and vanadium as essential for algal growth. He noted that nickel, titanium, selenium, lead, silver, gold, bromine, and iodine have been detected in microorganisms but have not yet been proven necessary for growth. Investigations of micronutrient limiting and inhibiting effects on natural phytoplankton production were reviewed by Goldman (1965).

Organic growth factor influence on production of natural phytoplankton populations has only recently been investigated. Fogg and Westlake (1955) determined that many organic solutes complex cations in natural waters, thus producing monovalent-to-divalent cation ratios more favorable for algal growth. Phinney and Peck (1961) found natural enrichment of Klamath Lake, Oregon promoted by the chelating effect of humic acids on low inorganic nutrient concentrations. Provasoli (1961) and Fogg (1965) reported that may algal species required vitamin B_{12}, thiamine, and biotin for growth; however, Provasoli (1963) considered it unlikely that vitamin B_{12} would often limit primary production in fresh waters. Menzel and Spaeth (1962) found that vitamin B_{12} influenced the species composition of a Sargasso Sea phytoplankton community, but did not limit primary production. Wetzel (1965a, 1965b) reported that phytoplankton growth in Indiana marl lakes was stimulated by additions of vitamin B_{12}, thiamine, biotin, ethylenediaminetetra-acetate (EDTA), citrate, glycolate, glycine, alanine, and tryptone. This was primarily due to the chelatory action of

these organic compounds, in addition to their function as sources of nitrogen, phosphorus, and carbon. Accumulation of dissolved organic matter, which occurs as a body of water becomes more productive, may increase inorganic nutrient availability by chelation and further accelerate eutrophication.

Sources of Nutrient Enrichment

Man's activities produce enormous quantities of wastes and have been the dominant factor increasing nutrient inflow to our water resources. Man-induced nutrient addition is often referred to as cultural enrichment, in contrast to natural conditions which presumably would exist in the absence of man (Sawyer 1968). Surface and ground water drainage from agricultural and urban areas, treated and untreated sewage effluent, and industrial waste discharge are major enrichment sources. Most aquatic fertilization studies have shown domestic and industrial wastewaters to be major nutrient contributors (Sawyer 1968).

Natural nutrient sources include surface and ground water runoff from undisturbed drainage basins, inflowing organic materials, fertilization by transient waterfowl, precipitation, and nutrient recycling from sediments and organic matter decomposition. Hutchinson (1957) related geochemical factors to phytoplankton standing crop in lakes. He observed that water bodies derived from sedimentary drainage were generally rich in phosphorus and highly productive, while those receiving drainage from metamorphic formations were nutrient-poor and less productive. Mackenthun, Ingram, and Porges (1964) and Fruh (1967) reviewed natural, agricultural, and urban drainage, the atmosphere, groundwater, and wastewater effluents as plant nutrient sources.

Inhibition of Eutrophication by Nutrient Control

Control of accelerated eutrophication has received major attention from water quality investigators in recent years. Most control methods are based on the assumption that primary production can be restricted by limiting critical nutrient availability. Oglesby and Edmondson (1966) and Sketelj and Fejic (1966) attempted to inhibit eutrophication in Green Lake, Washington and Lake Bled, Yugoslavia, respectively, by diluting them with nutrient-poor water. Localized eutrophication control by diversion of nutrient-rich inflow from a receiving body of water is effective (Thomas 1962; Oglesby and Edmondson 1966), but ultimately expands the problem by enriching water downstream (Mackenthun, Lveschow, and McNabb 1960). Olszewski (1961) successfully removed accumulated nutrients from a eutrophic lake in Poland by selective withdrawal of hypolimnetic water (Fruh 1967), but this method also leads to downstream enrichment (Knight 1965). Bryan (1965) found that artificial mixing of a stratified reservoir by aeration prevented the solution of many nutrients, and thus improved water quality. The most promising eutrophication control approach is direct nutrient removal by biological or chemical wastewater treatment (Martin and Weinberger 1966; Eliassen and Tchobanoglous 1968).

Determination of nutrients critical for algal growth is essential for evaluation of lake and reservoir water quality and the enriching potential of tributaries (Fruh 1967). Although the nutritive value of macronutrients, micronutrients, and organic growth factors is realized, removal of most of these elements is either impractical or ineffective (Fruh 1967; Sawyer 1968). Nitrogen occurrence in various nutrient forms (NH_4, NO_3, NO_2, and organic compounds) and the nitrogen-fixing ability of some blue-green algae make effective nitrogen removal impractical.

Phosphorus is usually considered the logical point of emphasis for eutrophication control, because it often limits aquatic primary production and effective removal techniques are available (Fruh 1967; Sawyer 1968).

DESCRIPTION OF THE STUDY AREA

Waco Reservoir (Fig. 1) is a 2942.2 hectare multi-purpose impoundment adjacent to the northwestern edge of Waco, McLennan County, Texas. The earthen embankment dam is located on the Bosque River approximately five river miles above its confluence with the Brazos River. The reservoir was constructed for flood control and water storage, and provides the primary water supply for the Waco metropolitan area. Morphometric characteristics of the impoundment are listed in Table 1.

Waco Reservoir receives drainage from four major tributaries: the North, Middle, and South Bosque Rivers, and Hog Creek. The North Bosque River enters the reservoir west arm, and contributes 70 percent of the annual tributary inflow (calculated from 1968 U.S. Geological Survey records). Hog Creek, the Middle Bosque, and South Bosque Rivers drain into the south arm (Fig. 1). These tributaries drain a 427,520 hectare basin of limestone and shale formations.

The present impoundment is the second on the same site. The first reservoir was constructed in 1930 to provide a municipal water supply for Waco. High sedimentation rates decreased the original storage capacity approximately 50% by 1947 (Fig. 2). The reservoir had become a dystrophic marsh by 1960, and the reduction of water quality and quantity made necessary the use of Brazos River water to supplement the municipal supply. The U.S. Army Corps of Engineers completed a second and larger dam just downstrean from the first in February, 1965. Heavy rains filled the new reservoir to conservation pool level that spring.

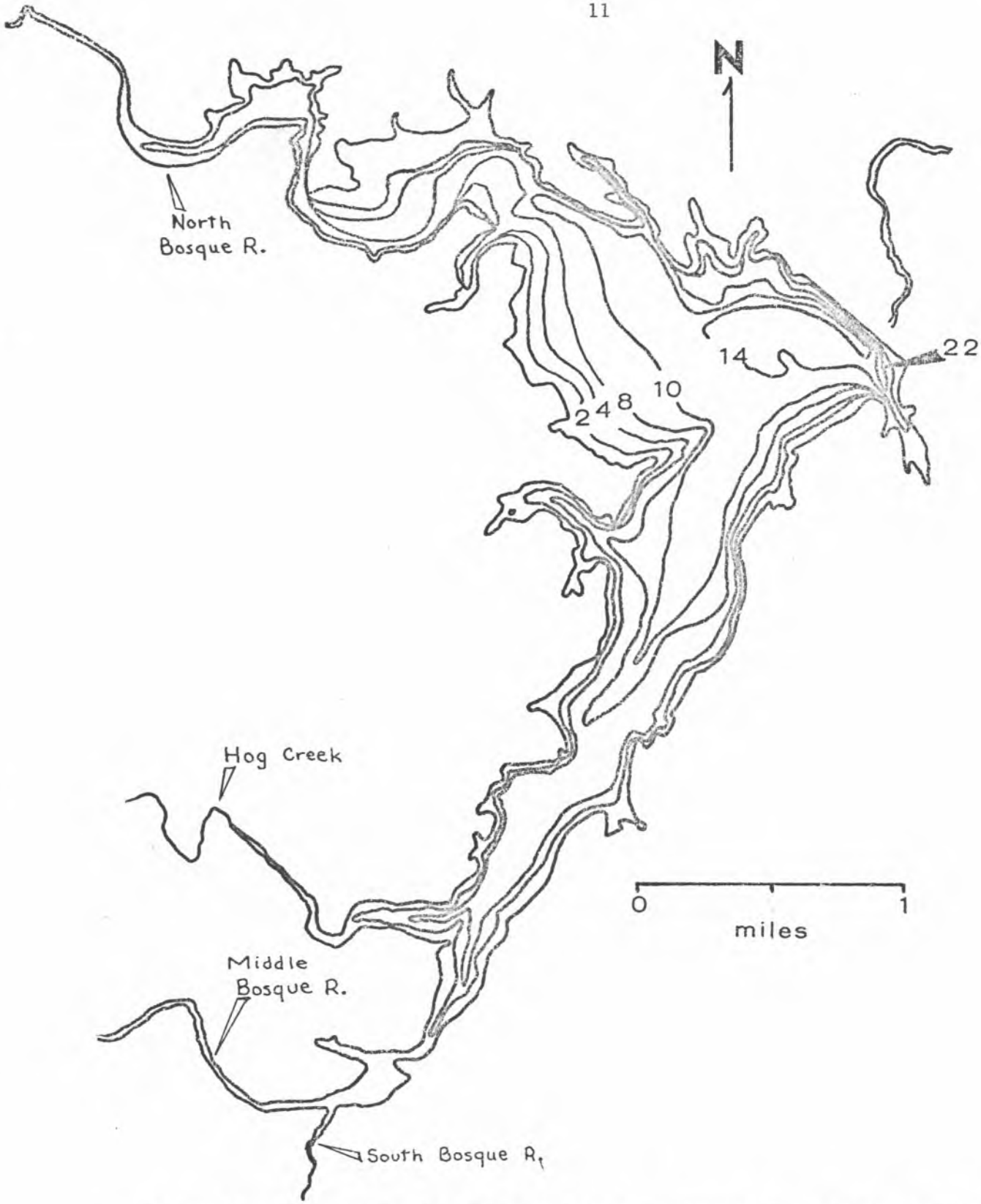

Figure 1. Waco Reservoir and tributaries, McLennan Co., Texas. Depth contours in meters.

Table 1. Morphometric characteristics of Waco Reservoir at normal conservation pool level.

Mean sea level elevation	184 m
Surface area	2942 ha
Volume	127.36 (10^6 m^3)
Maximum depth	22 m
Mean depth	4 m
Length of shoreline	96.6 km
Shoreline development	5.02
Watershed area	427,520 ha
Watershed to surface area ratio	145 to 1

Figure 2. Reduction of old Waco Reservoir storage capacity between 1930 and 1947. Data from Jones and Rogers (1952) in Spencer (1966).

The new Waco Reservoir is subject to greater forces of eutrophication than the old impoundment. Improved soil conservation practices in the drainage basin and the increased total storage capacity to drainage area ratio of the new reservoir should reduce the former excessive rate of storage capacity loss by sedimentation (Spencer 1966). However, the new reservoir is subject to more nutrient enrichment by cultural drainage than the old Lake Waco. Some nutrient inflow already reaches the reservoir as treated sewage and industrial wastes released into tributaries of the South Bosque River, treated sewage effluent entering the North Bosque River, and agricultural drainage from the entire Bosque River basin. Septic tank seepage from suburban areas near the impoundment provides another nutrient source. Waco Reservoir is threatened by progressively higher degrees of cultural enrichment and accelerated eutrophication as the Waco metropolitan area expands around it.

METHODS

Field Procedures

Three stations were established to determine the physical, chemical, and biological characteristics of Waco Reservoir. Sampling stations were located in each of the two main arms and in the reservoir main body (Fig. 3). Water was sampled and primary production measured at weekly to bi-weekly intervals from June to November, 1968.

Water Sampling and Physical Measurements

Water samples were collected with a two-liter plastic Kemmerer water bottle. Immediately after collection, all samples were stored on ice in blackened insulated boxes until return to the laboratory. Water temperature and relative light penetration were measured in situ with a portable thermistor unit and a Whitney submarine photometer, respectively. Nannoplankton was collected at the 50% surface illumination level (usually about 0.5 m) and immediately preserved in 5% formalin. Net plankton was collected via three 6 m vertical hauls with a #20 Wisconsin plankton net and similarly preserved.

Primary Production Measurement

The carbon-14 (^{14}C) isotope method of Steeman Nielsen (1951, 1952) as modified by Goldman (1963) and Lind (1966), was used to measured net phytoplankton production. This method is an isotope dilution technique based on the assumption that biological uptake of ^{12}C and ^{14}C is

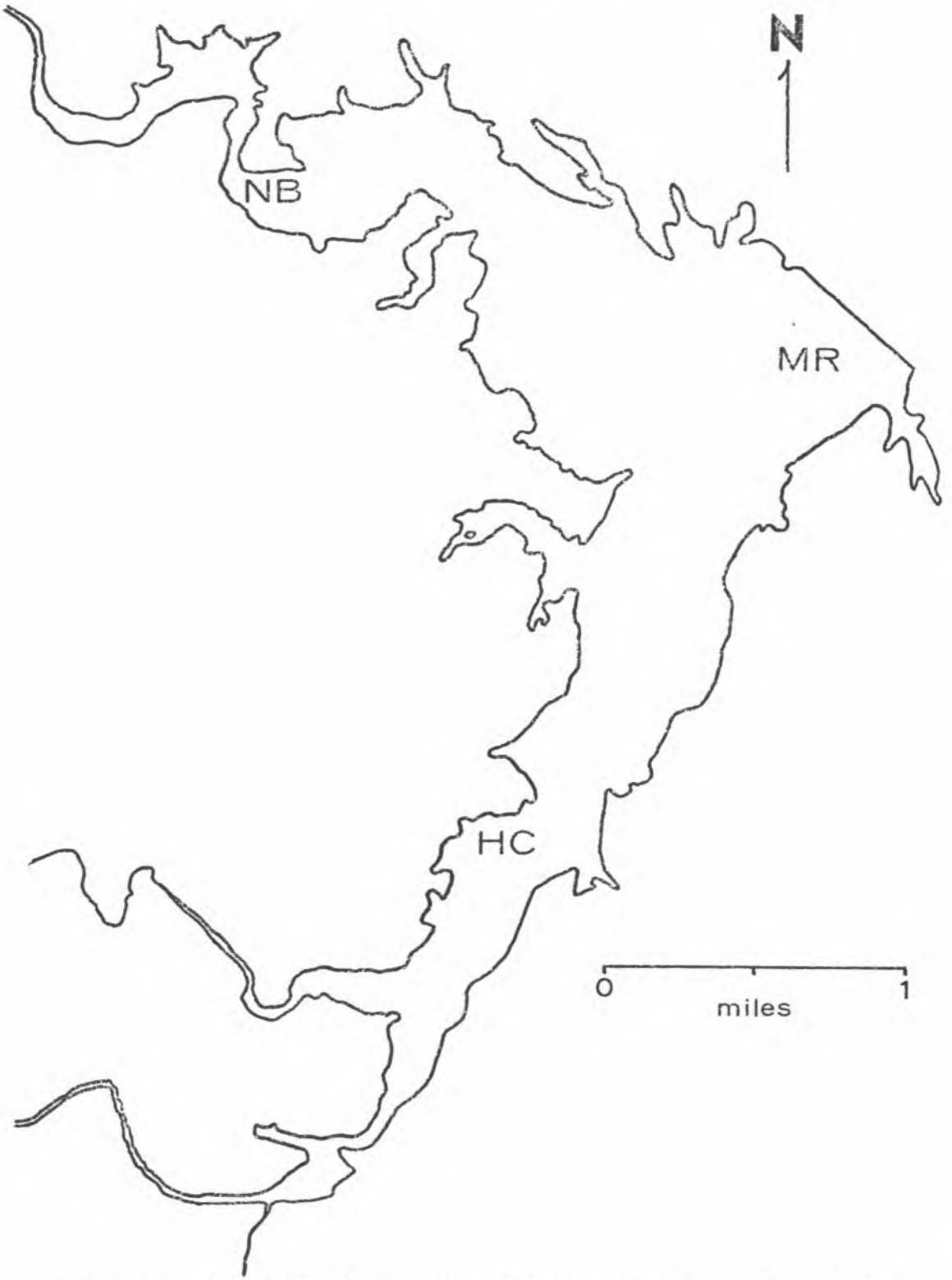

Figure 3. Location of sampling stations in the Hog Creek arm (HC), the North Bosque arm (NB), and the main body of Waco Reservoir (MR).

proportional to their environmental concentrations.

Water samples were collected from the surface and depths receiving 75, 50, 25, and 1% surface illumination and placed in 125 ml glass-stoppered pyrex bottles. Samples from each station were stored in an insulated box, blackened interiorly to prevent light entrance, while remaining stations were sampled. Environmental sample temperature was maintained by filling the box with lake water. Total sampling time was less than two hours. At MR, samples for dark fixation determinations were taken from depths corresponding to each illumination level and placed in 125 ml dark bottles, foil-wrapped for heat reflection. Dark fixation at MR was assumed to approximate that at HC and NB.

After all samples had been collected, they were shaken to insure a homogeneous plankton suspension and 1 ml pipetted from each. One ml $Na_2{}^{14}CO_3$ solution (2.33 microcuries/ml)[1] was injected into each sample with a 4-inch, 14 gauge laboratory cannula and a Hamilton gas-tight syringe with a Chaney adaption[2], allowing a precision of delivery of $\pm$ 0.01%. Each bottle was stoppered immediately after inoculation to prevent loss of $^{14}CO_2$. All samples were kept shaded in the blackened box to avoid direct exposure to surface light during inoculation. Light inhibition significantly reduces carbon fixation in samples taken from depths and exposed to direct surface light (Goldman, Mason, and Wood 1963). After inoculation, all samples were snapped into their appropriate positions on an aluminum incubation rack (Fig. 4, Appendix A) and lowered into the water. All samples were incubated *in situ* at MR at the illumination level from which they were taken. Photic zone water temperatures were equivalent at all stations, so *in situ* incubation closely approximated environmental

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[1]New England Nuclear Corp.; Boston, Massachusetts.

[2]Hamilton Co.; Whittier, California

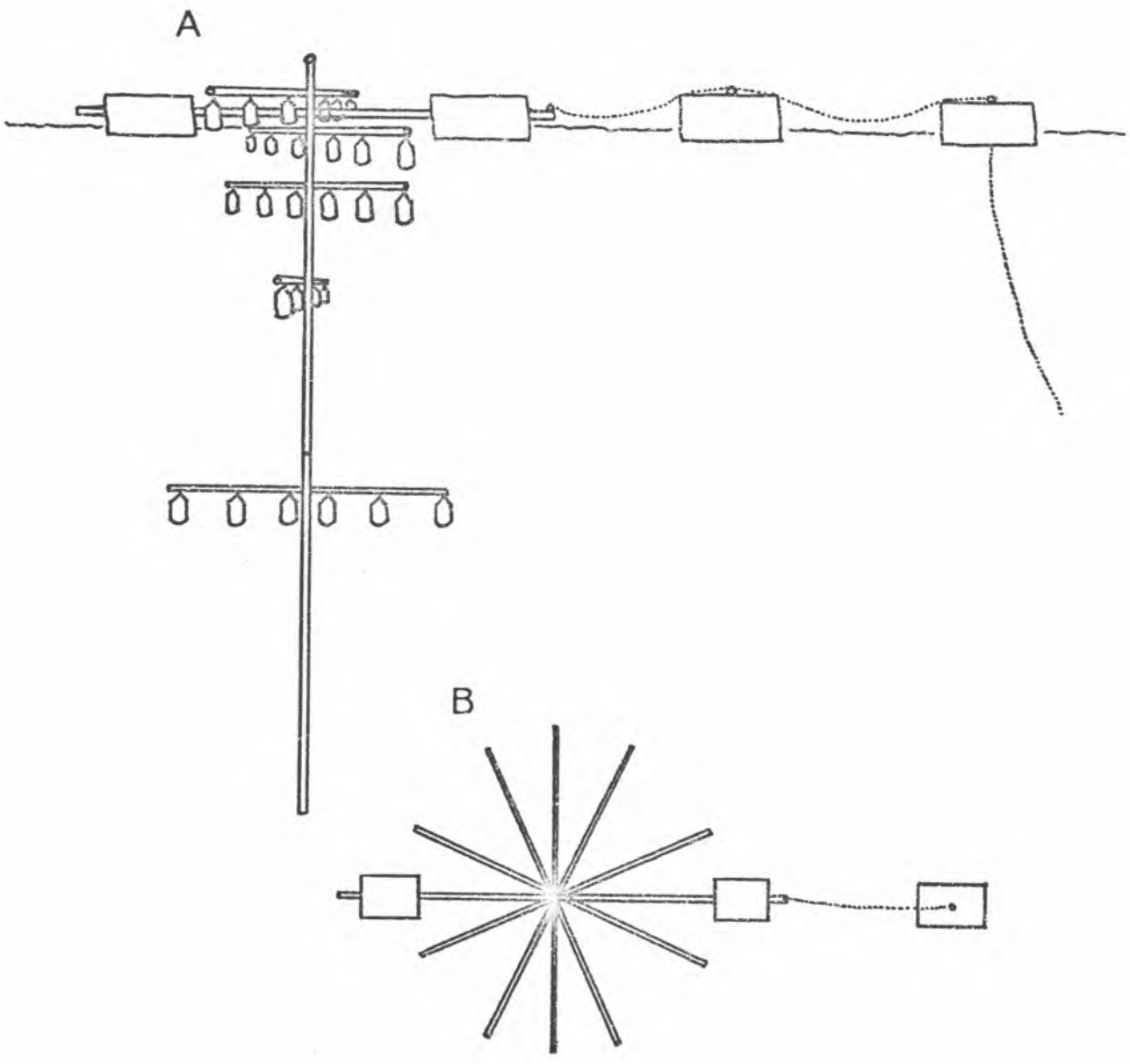

Figure 4. Diagram of assembled incubation rack in water (A). Top view (B) shows horizontal supports radially arranged to avoid sample shading.

conditions. The samples were routinely suspended from 10 A.M. to 2 P.M. Central Standard Time. A four hour incubation period is long enough for adequate ^{14}C uptake, yet sufficiently brief to avoid deleterious bottle effects (Vollenweider and Nauwerck 1961). Samples were then retrieved, iced down in the blackened insulated box to minimize further carbon fixation, and returned to the laboratory for immediate filtration. No more than two hours elapsed between sample retrieval and filtration. Lind (1966) demonstrated that short delays in filtration had no significant effect on sample activity.

Determination of Nutrient Limiting Factors and Artificial Enrichment Effects

Classical approaches to relating nutrient levels to aquatic primary production have involved correlations of physical and chemical parameters to phytoplankton growth fluctuations. However, measurements of instantaneous environmental nutrient concentrations are of questionable ecological significance, because they may reflect only nutrient levels maintained by a dynamic equilibrium between organisms, water, and sediments. Several investigators have shown that algae indulge in "luxury consumption" and store essential nutrients in excess of immediate requirements in a plentiful nutrient supply (Lund 1950; Gerloff and Skoog 1954). Chemical analyses yield information on nutrient quantities present which are reactive with the analytical reagents used, but may not indicate the actual nutrient availability for primary production (Gerloff and Skoog 1954; Potash 1956; Ryther and Guillard 1959; Kuenzler and Ketchum 1962; Menzel, Hulbert, and Ryther 1963; Fogg 1965; Wetzel 1965; and Rigler 1966 and 1968.

In situ bioassay methods are more direct measurements of producer nutrient requirements. Goldman (1960a, 1960b, and 1964), Schelske (1962),

Goldman and Wetzel (1963), Wetzel (1964, 1965), and Goldman and Carter (1965) used *in situ* radiocarbon bioassay techniques in fresh water. Carbon fixation in experimentally enriched samples was compared to that in control samples to determine nutrient deficiencies. Carbon-14 techniques provide a convenient and accurate means for field determination of nutrient limiting factors and enrichment effects, because of the isotope method sensitivity (Wetzel 1964; Goldman and Carter 1965). *In situ* ^{14}C bioassay techniques as described by Goldman (1960a, 1960b, 1962) and Goldman and Carter (1965) were used in this study.

Various methods have been used to isolate water masses for *in situ* production studies. Most techniques involve complete enclosure of the water mass in a glass vessel, plastic bag (Strickland and Terhune 1961), or plastic sphere (McAllister et al. 1961; Antia et al. 1963). Complete enclosure introduces complicating unnatural circulation or lack of circulation (Antia et al. 1963). A better method is to use open-ended cylinders allowing water column isolation from the surrounding environment, but avoiding disruption of natural chemical, physical, and biological stratification. Such an apparatus first appeared as the small diameter (5.4-5.6 cm) plexiglas cylinder of Thomas (1958) and more recently as the large diameter (0.5-1.0 m) polyethylene cylinder of Goldman (1962).

Water columns, isolated by polyethylene film, (Fig. 5) were artificially enriched with NaH_2PO_4 solution at each station during June and July, 1968. The phosphate solution concentration was such that the solution's uniform distribution in the isolated water column resulted in an addition of 0.03 mg PO_4-P l^{-1} water. Polyethylene cylinder construction and uniform nutrient addition to isolated water columns followed Goldman (1962), and are described in Appendix B.

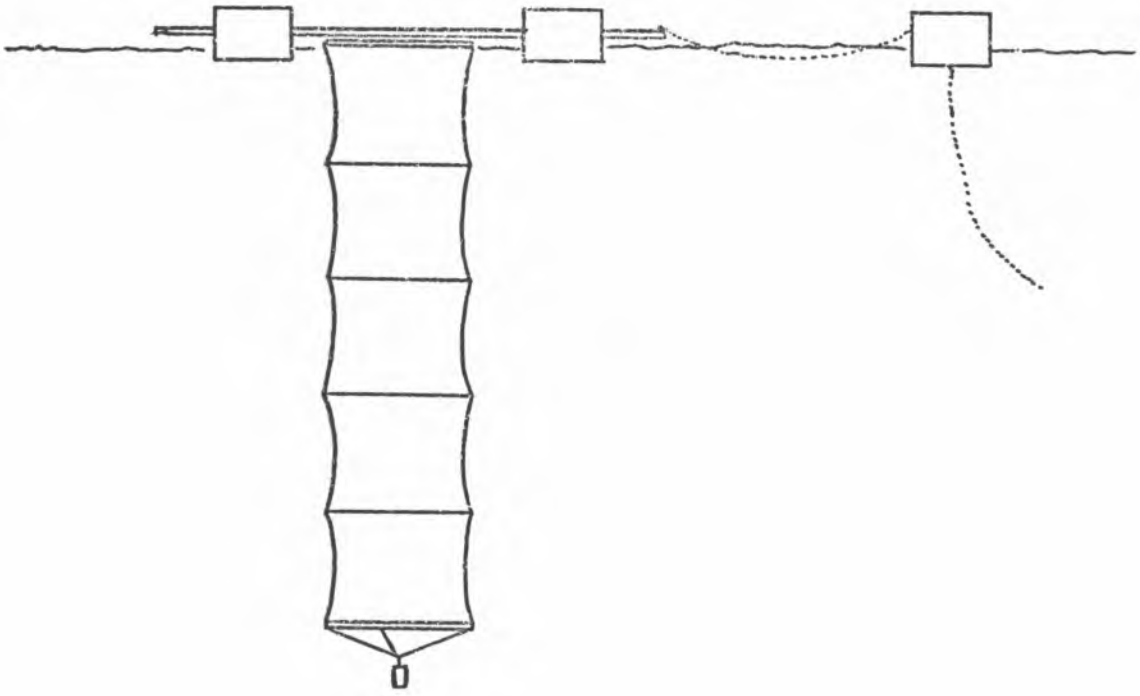

Figure 5. Diagram of polyethylene cylinder as used to isolate a water column for experimental nutrient enrichment.

An effort was made to identify additional nutrient factors influencing phutoplankton production in Waco Reservoir during August and September, 1968. The field procedure was modified to that of Goldman (1960a). Eight replicate water samples were collected at each station from the 50% illumination level and 2 ml pipetted from each. Each sample was inoculated with 1 ml of a nutrient solution, 1 ml of $Na_2{}^{14}CO_3$ solution, and incubated in situ for four hours. Eight nutrient solutions were used with one sample from each station being inoculated with a different nutrient additive and incubated as described above. The addition of one ml of nutrient solution to a 125 ml sample increased nutrient concentration in proportion to its approximate environmental level (Table 2). Added nutrients must be in very low concentrations, similar to those present in nature, if experimental enrichment is to yield meaningful data (Verduin 1964).

A combination of nutrients (Si, Fe, PO_4) which were suspected to stimulate phytoplankton growth were added to isolated water columns during October and November, 1968. Reagents and concentrations used were as described in Table 2. Carbon fixation in enriched samples was compared to that in unenriched samples to determine nutrient enrichment effects on phytoplankton production.

Table 2. Composition of nutrient solutions used in rapid ^{14}C bioassay for limiting nutrients in Waco Reservoir.

Nutrient solution	Reagent used	Concentration of stock nutrient solution (g-ion l^{-1})	Nutrient Concentration after inoculation (mg l^{-1})	Environmental Concentration range (mg l^{-1})
Nitrate	KNO_3	0.25	2.5	2
Phosphate	NaH_2PO_4	0.0125	0.0125	0.005-0.02
Iron	$FeSO_4$-Na_2(EDTA)	0.050	0.50	0.2
EDTA	Na_2(EDTA)	0.05	0.5	-
Sulfate	$CaSO_4$	5.0	50.	20.-35.
Silica	Na_2SiO_3	1.0	10.	6-10
Micronutrients:				
Molybdenum	$Na_2MoO_4 \cdot 2H_2O$	0.001	0.01	-
Manganese	$MnCl_2 8$	0.001	0.01	-
Magnesium	$MgCl_2$	0.001	0.01	-
Zinc	$ZnSO_4$	0.001	0.01	-
Copper	$CuSO_4$	0.001	0.01	-
Cobalt	$CoCl_2$	0.001	0.01	-
Boron	H_3BO_3	0.001	0.01	-
Complete	(above)	-	(as above)	-

Laboratory Procedures

Analysis of Water Samples

Water samples were refrigerated until analysis. Routinely, all analyses were completed within 48 hrs. of the sampling time. Standard analytical methods (APHA 1965) were used to determine dissolved oxygen, pH, total alkalinity, specific conductance, and silica. Hach[3] procedures were used to determine NO_3-N and SO_4 concentrations. The bipyridine method (Rainwater and Thatcher 1960) was used to determine total iron. Turbidity was determined photometrically at 450 mμ and percent transmittance values were converted to Jackson turbidity units using a Hach table based on a standard formazin solution.

The single solution phosphomolybdate extraction method of Murphy and Riley (1962), as modified by Stephens (1936), was used to determine PO_4-P concentrations. Absorbance of the molybdemum blue complex was determined at 830 mμ to improve sensitivity of the method (E. J. Griffith, personal communication). The extreme sensitivity of this method required considerable caution to avoid sample contamination. All glassware used in sample collection and analysis was soaked for 14 days in a 1% HF-2N HCl solution to prevent anion adsorption to glass surfaces as described by Hassenteufel, Jagitsch, and Koczy (1963). No detergent was used in glassware washing at any time. Glassware was rinsed three times with tap water and glass-distilled water immediately before use. After use, glassware was rinsed three times with tap water, once with concentrated H_2SO_4, and stored in a dilute HCl bath. Four 1.6 μg PO_4-P l^{-1} standard solutions were analyzed to test the method's reproducibility. Mean optical density was 0.086± 0.001 (SD). All photometric determinations were made with a

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[3]Hach Chemical Co.; Ames, Iowa.

Bausch and Lomb Spectronic 20 colorimeter through a 2.5 cm light path.

Plankton Identification and Enumeration

Net plankton and nannoplankton were identified and quantitatively enumerated by microscopic examination in a 1 ml Sedgewick-Rafter counting cell (APHA 1965). Nannoplankton samples were concentrated prior to counting by centrifugation. Phytoplankton identification was made using Smith (1950) and Edmondson (1963).

Primary Production Measurement

Sample Filtration and Preparation

All samples were filtered immediately upon return to the laboratory. A 100 ml aliquot of each sample was filtered through a 47 mm 0.45 u Millipore[4] membrane filter at 100 mm Hg vacuum. Very low filtration pressure was used to avoid assimilated ^{14}C loss by rupturing algal cells in filtration. Guillard and Wangersky (1958) and Arthur and Rigler (1967) noted significant loss of fixed carbon through the filter when high filtration pressure was used. Following sample filtration, the filter was rinsed with 10 ml of 0.003 N HCl to eliminate carbonate precipitation (Goldman 1963). This procedure was necessary because of the alkaline nature of Waco Reservoir water. Acid treatment is usually unnecessary in fresh waters except in conditions of high pH where carbonate precipitation may occur (Rodhe, Vollenweider, and Nauwerck 1958). Wetzel (1965) found that significant error in primary production determinations may result if filters are not decontaminated. Exposure of filtered samples to fuming HCl was recommended by Steeman Nielsen (1952) and Wetzel (1965). Strickland (1960) and McAllister (1961) suggested that errors due to

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[4]Millipore Filter Corp.; Bedford, Massachusetts.

decontamination procedures may exceed those caused by contamination. Filters were rinsed with 50 ml deionized water following the weak acid rinse, to wash down cells adhering to the filtration funnel and to remove excess acid. Residual acid sometimes reacted with the liquid scintillation fluor solution to produce an opaque green coating on the filter. This did not occur if acid was thoroughly rinsed from the membrane filter.

The damp filter was placed in a glass screw-cap vial, so the filter lined the inner wall with its algal coating facing the inside of the vial. Samples were desiccated over silica gel for at least 48 hrs. Indicarb[5] was included in the desiccator to absorb atmospheric CO_2 and thus minimize isotope exchange. After drying, 20 ml scintillation fluor solution were added to each and the vials capped immediately to prevent water absorption. The fluor solution was 4.0 g 1,5-diphenyloxazole (PPO) and 100 ml 1,4-bis-2 - (5-phenyloxazolyl)-benzene (dimethyl-POPOP) dissolved in one liter reagent grade toluene.

Liquid Scintillation Counting

Sample radioactivity was determined by triplicate ten minute counts in an automatic Beckman LS-100 liquid scintillation unit[6]. Blank samples containing filter and fluor solution were counted to determine background activity. Backgound counts were subtracted from each sample count. Efficiency of the Beckman unit was determined to be 90.6% by counting a Beckman toluene-^{14}C standard.

Liquid scintillation theory and techniques were presented by Chase and Rabinowitz (1959) and Wang and Willis (1965). The counting efficiency

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[5]Fisher Scientific Co.

[6]Beckman Instruments, Inc.; Fullerton, California

of a liquid scintillation system is most affected by quenching activity of sample-fluor solution components. Quenching is any process which reduces fluorescent energy transfer efficiency in the scintillation system (Wang and Willis 1965). Quenching occurs in all liquid scintillation sample-fluor solutions, and its extent must be determined for quantitative radioactivity measurements. The fluor solution, algal cells and silt particles filtered from the water sample, and the membrane filter produced quenching in this study. To determine the extent of this quenching, progressively greater quantities of Waco Reservoir water were Millipore filtered, the filters dried, and placed in counting vials with 20 ml fluor solution. These samples were counted for 10 min. each in triplicate to determine a mean background count for each sample. One-tenth milliliter (44166 dpm) standardized toluene-^{14}C[7] was added to each vial with a 0.2 ml capacity micrometer buret[8], the samples counted, and the counts corrected for background. The results (Table 3) indicated that quenching by materials filtered from Waco Reservoir water resulted in only two or three percent reduction in counting efficiency.

Liquid scintillation techniques hold distinct advantages over Geiger-Mueller (G-M) and gas phase radioactivity determinations traditionally used in radiocarbon primary production measurements. The detection of activity of low energy beta emitters, such as ^{14}C, is greatly enhanced by the intimate sample-fluor relationship. Corrections for variable geometry, coincidence loss, window absorption, self absorption, and backscattering encountered in G-M assay are unnecessary with liquid scintillation systems (Wang and Willis 1965). Liquid scintillation sample preparation is simpler than preparation of planchet-mounted

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[7]Packard Instruments Co., Inc.; Downers Grove, Illinois.

[8]Roger Gilmont Instruments, Inc.; Great Neck, New York.

Table 3. Quenching effect of materials filtered from progressively greater quantities of Waco Reservoir water and suspended on 47 mm Millipore filters.

Net counts/sec. ($\bar{x} \pm SD$)	Volume filtered (ml)	% Counting Efficiency
666.04 ± 1.32	0	90.5
658.41 ± 1.23	15	89.4
660.08 ± 1.62	25	89.7
663.07 ± 0.33	50	90.1
653.18 ± 0.92	75	88.7
662.85 ± 0.66	100	90.0
658.30 ± 1.32	125	89.4
660.76 ± 0.24	150	89.8
652.76 ± 1.23	200	88.7

samples for G-M determinations, or individual combustion of samples for gas phase counting. Another advantage of liquid scintillation counting is that counting efficiency may be determined on the same instrument, making duplication of conditions with other ^{14}C-labeled materials unnecessary (Wolfe and Schelske 1967). Automatic scintillation units allow the counting of a large number of samples with minimum effort. Jitts and Scott (1961) and Wolfe and Schelske (1967) used liquid scintillation techniques to determine Geiger counting efficiency. Olson and Putnam (unpublished) and Lind (1966) directly applied liquid scintillation counting to primary production measurements with ^{14}C, as was done in this investigation.

Experimental Error of ^{14}C Method

Carbon fixation in replicate MR samples was measured to estimate the experimental error of ^{14}C primary production determinations in Waco Reservoir. Each water sample was collected individually to prevent artificial reduction of variation between samples. Three light bottles and one dark bottle were suspended at each of five depths. Inoculation, incubation, laboratory preparation, and scintillation counting of samples were as described above. Data were used to estimate the experimental error in measurements of integral carbon fixation below a unit area (m^2) of water surface, as described by Goldman and Carter (1965) and explained in Appendix C. Integral net carbon fixation was 268.7 counts sec^{-1} m^{-2} with a variance of 151.2 counts sec^{-1} m^{-2} and a standard deviation of 12.3 counts sec^{-1} m^{-2}. The standard deviation was 4.6% of the integral net carbon fixation. This experimental error was lower than those (34.4%, 15.7%) reported by Goldman and Carter (1965) for ^{14}C primary production measurements in Lake Tahoe. The low

productivity of Lake Tahoe caused measurements there to be less precise than in Waco Reservoir. Doty (unpublished) noted a reduction in ^{14}C method precision when measured production was low.

Most ^{14}C experimental error estimates are expressed as coefficients of variation (V):

$$V = 100\frac{S}{M}\%$$

where S = the standard deviation of a series of replicate measurements, and M = the arithmetic mean of these measurements. Coefficients of variation calculated from these data for each depth sampled ranged from 3 to 11% and varied inversely with the measured production (Fig. 6). Steeman Nielsen (1952) and Doty (unpublished) calculated V = 5.8% and 11% respectively, for marine primary production measurements. Cassie (1963) reported that V = 10% represents the minimum error achievable by routine techniques in marine studies.

Calculation of Primary Production

Sample radioactivity is proportional to carbon assimilation. The formula of Saunder, Trama, and Bachman (1962) was used to calculate carbon assimilation (see Appendix D). Dark bottle samples were incubated simultaneously with illuminated samples to determine non-photosynthetic carbon assimilation. Photosynthetic carbon assimilation was then calculated as:

$$P_{net} = P_{light} - P_{dark}$$

Where: P_{net} = net photosynthetic carbon fixation,

P_{light} = light bottle carbon fixation, and

P_{dark} = dark bottle carbon fixation.

Standard corrections for dark uptake have been used (Steeman Nielsen 1952; Ryther and Vaccaro 1954), but more recently variation in dark fixation has been shown (Steeman Nielsen and Al Kholy 1956; Currie 1958; and Steeman Nielsen 1960). Most workers incubate samples in both dark

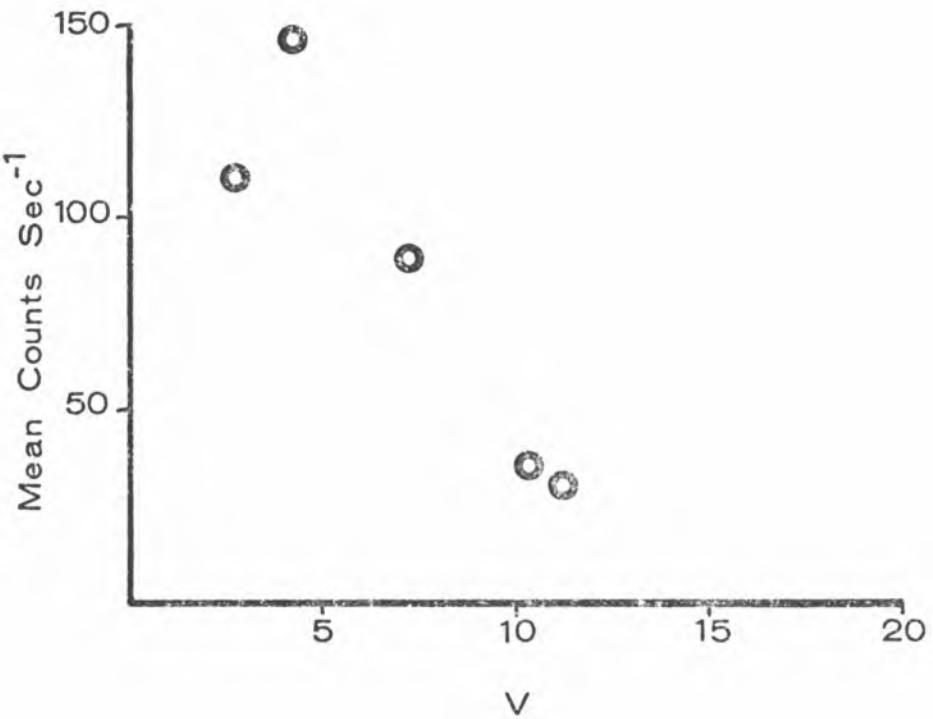

Figure 6. The inverse relationship of the coefficient of variation (V) to relative carbon fixation in Waco Reservoir samples.

and light bottles to provide an empirical correction for non-photosynthetic carbon assimilation.

Calculation of Daily Photosynthesis and Photosynthetic Efficiency

Daily net phytoplankton production estimates were calculated from values determined for mid-day 4-hour incubation periods. Values for primary production during mid-day in situ incubation at each illumination level were plotted against depth and integrated by planimetry. Integral four-hour estimates were converted to values for daily production per square meter using a 2.5 multiplication factor. This factor was based on the reference integral method of Vollenweider (1965), and is similar to previously used conversion factors, which range from 2 to 3 (Rodhe, Vollenweider, and Nauwerck 1958; Vollenweider and Nauwerck 1961; and Vollenweider 1965). Daily phytoplankton production per unit volume of water in the photic zone was calculated by dividing production per square meter by the photic zone depth. The photic zone was considered to extend to the depth of 1% surface light intensity. It was assumed that any photosynthetic production occurring below this depth was negligible.

Eppley pyrheliometer values recorded on each sampling date at Ft. Worth and San Antonio U.S. Weather Bureau stations were averaged to estimate solar radiation at the Waco Reservoir water surface. Waco is the approximate midpoint between Ft. Worth and San Antonio, so this average provided reasonable estimations. Photosynthetic efficiency of light energy utilization was calculated as follows:

$$\text{Efficiency (\%)} = \frac{(\text{mg C m}^{-2}\ \text{day}^{-1})\ (2)\ (5.5)}{(\text{g-cal m}^{-2}\ \text{day}^{-1})\ (0.5)}$$

Where: 2 = converts carbon to dry organic matter

5.5 = caloric content of phytoplankton organic matter (5.5 kcal g^{-1})

0.5 = converts total solar radiation to photosynthetically active radiation (Edmondson, 1956).

Data Analysis

Analysis of variance was used to test differences in physical, chemical, and biological variables between stations and between sampling dates. Experimental nutrient enrichment and light inhibition effects on phytoplankton production at each station were also tested by analysis of variance. A fixed-effects randomized block design model (Kirk 1968) was used, because (a) water samples randomly collected from the same location at approximately the same time could be considered matched samples with equal variances, (b) samples collected on different dates could not be considered random samples from a single population, and (c) the nuisance variable of individual differences between sampling dates was thus minimized. The experimental data satisfied assumptions associated with statistical testing based on the F distribution and randomized block design (Kirk 1968). Linear and partial correlation analyses (Steel and Torrie 1960) were used to test environmental variables and phytoplankton production relationships.

RESULTS

Waco Reservoir was found to be a highly productive, eutrophic body of water. Steady winds continuously mixed the reservoir and prevented thermal stratification (Fig. 7). Particulate inflow from the watershed, silt particles suspended by constant mixing, and high phytoplankton standing crops kept the water turbid. Statistical analysis revealed considerable variability in physical, chemical, and biological characteristics of different reservoir areas.

Comparison of Reservoir Areas

Physical and Chemical Factors

Light Extinction

Important differences in optical properties of water at the three sampling sites were found. Turbidity at each station varied directly with tributary inflow. Monthly precipitation and surface drainage into the reservoir decreased photic depth (Fig. 8). Hog Creek arm (HC) was significantly more turbid ($P = 0.05$)[9] than the North Bosque arm (NB) and the main body of the reservoir (MR). Although light extinction was consistently greater at NB than at MR (Table 4), there was no significant difference.

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[9]All statements of statistical significance refer to the stated level of probability for the appropriate degrees of freedom. Refer to Appendix for summary of analysis.

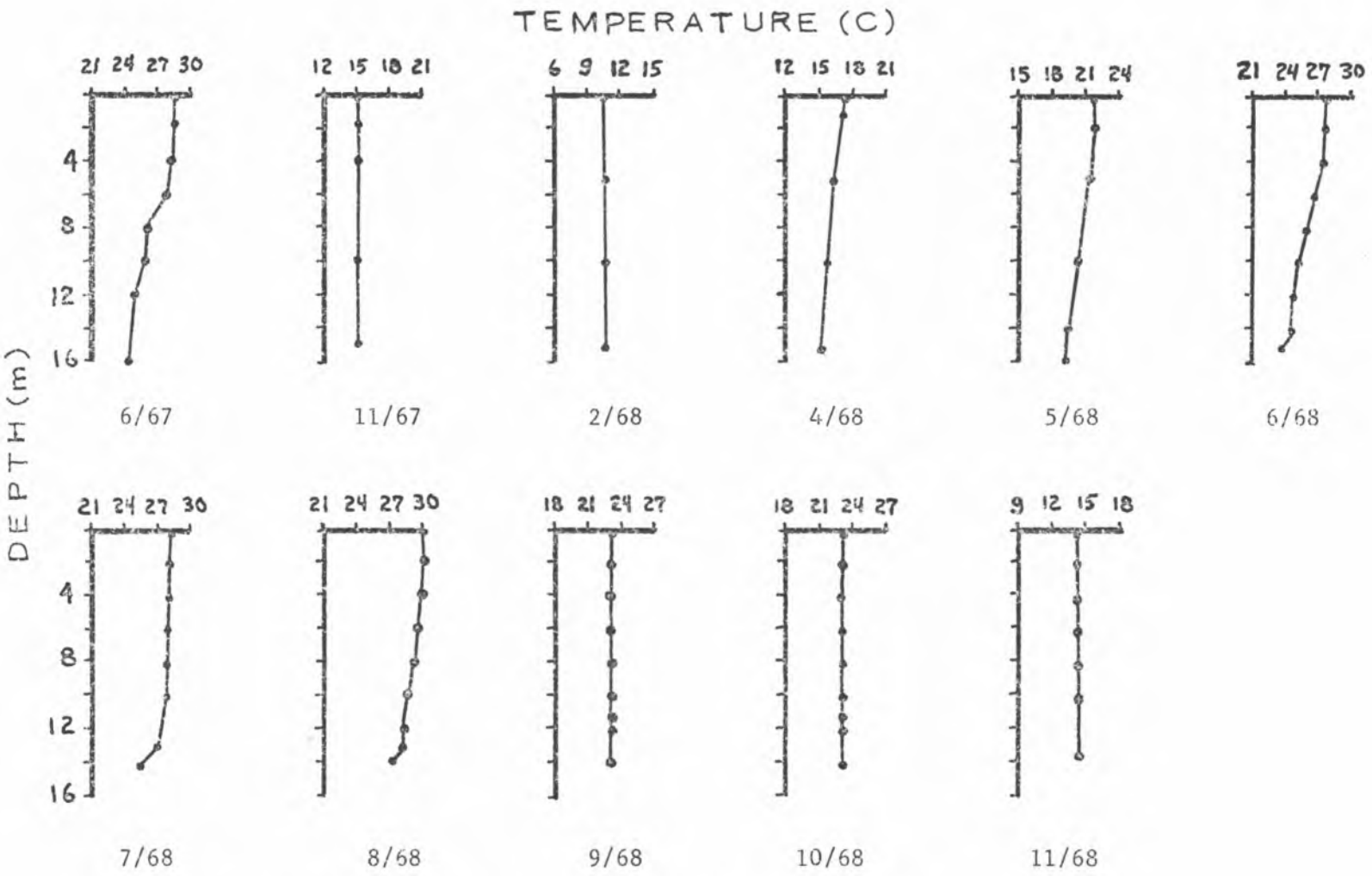


Figure 7. Selected temperature profiles of the main body of Waco Reservoir.

Figure 8. Effect of local monthly precipitation and surface drainage on photic depth in the main body of Waco Reservoir, 1968.

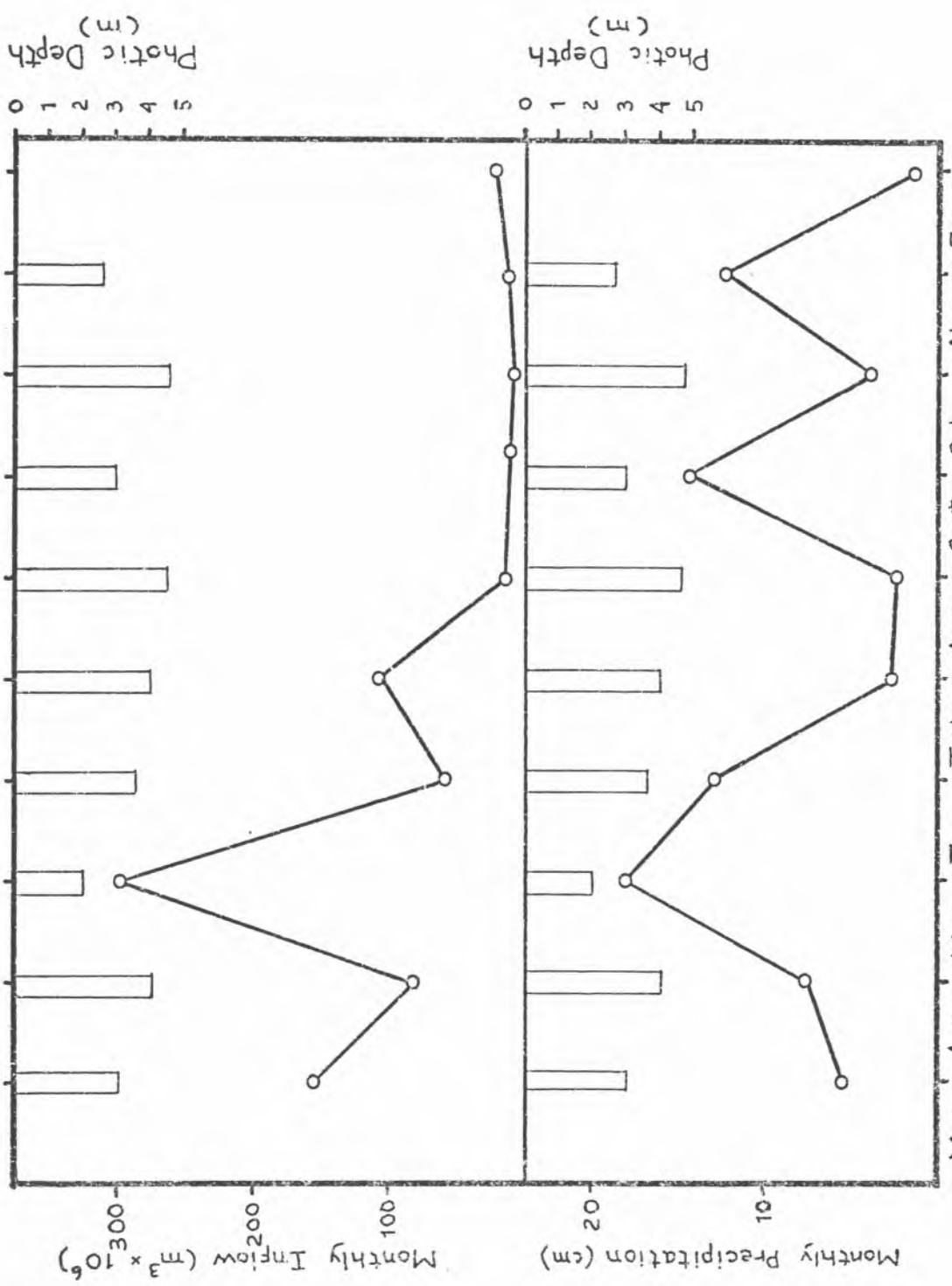

Monthly Precipitation (cm)
Monthly Inflow ($m^3 \times 10^6$)
Photic Depth (m)
Photic Depth (m)
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov
Dec

Table 4. Optical characteristics of Waco Reservoir stations.

	Turbidity (J.T.U.*)		Light Extinction Coefficient		Photic Depth (m)	
	Annual Mean	Range	Annual Mean	Range	Annual Mean	Range
Hog Creek Arm	64	49-95	2.37	1.42-3.09	1.8	1.0-3.2
North Bosque Arm	45	16-61	1.94	1.42-2.56	2.1	1.0-3.2
Main Lake	44	26-80	1.42	0.87-2.52	3.3	2.0-5.3

*Jackson turbidity units

Chemical Conditions

The reservoir was uniformly alkaline and well-buffered. Total alkalinity, pH, and free CO_2 concentrations were approximately the same at all three stations. Mean nutrient concentrations were generally higher in the reservoir arms than at MR (Table 5). Concentrations of PO_4-P at MR were lower than those at NB ($P = 0.05$) and HC ($P = 0.10$). Both tributaries also had higher SO_4 concentrations ($P = 0.10$) than the reservoir main body. Phosphate-phosphorus and sulfate concentrations between tributaries were not significantly different. Differences in silica content between stations were not significant.

Standing Crop and Primary Production

Phytoplankton samples taken from August to November, 1968 indicated that standing crop fluctuated drastically between sampling dates (Fig. 9). Late August Synedra and Tetraedron blooms at MR and a Synedra bloom at NB in early September bias mean phytoplankton standing crop values for the three stations. Adjusted averages corrected for bloom conditions (by discounting the bloom organism) gave a more accurate representation (Table 6), and indicated that average phytoplankton standing crop was greater in the reservoir main body than in the tributary arms. Four diatom genera composed varying proportions of the total phytoplankton standing crop in different reservoir areas. Diatoms composed a greater part of the standing crop at HC than at NB and MR (Table 7); however, analysis of variance indicated no significant differences in total phytoplankton or diatom standing crop between the three stations.

Phytoplankton production was high in all reservoir areas and fluctuated in response to previous tributary inflow. Highest primary production

Table 5. Chemical characteristics of Waco Reservoir stations. Values are means with observed ranges in parentheses.

Variable	Hog Creek Arm	North Bosque Arm	Main Lake
Specific conductance (micro-mhos at 18 C)	290 (240-325)	310 (250-340)	290 (225-330)
pH	(8.0-9.3)	(7.9-8.9)	(8.1-9.1)
Alkalinity (mg $CaCO_3$ l^{-1})	148 (129-161)	156 (112-176)	145 (107-162)
Free CO_2 (mg l^{-1})	1.01 (0.46-2.35)	1.07 (0.46-3.22)	1.05 (0.44-1.88)
Phosphate-phosphorus (mg l^{-1})	2.7 (0-5.5)	2.6 (0.7-5.9)	2.0 (0-7.2)
Nitrate-nitrogen (mg l^{-1})	0.4	0.2	<0.2
Silica (mg l^{-1})	8.7 (7.2-10.6)	9.1 (7.0-10.6)	7.9 (5.8-10.0)
Total iron (mg l^{-1})	0.2	<0.2	<0.2
Sulfate (mg l^{-1})	29 (24-36)	27 (22-32)	25 (21-32)

Figure 9. Phytoplankton standing crop fluctuations in the Hog Creek arm (HC), the North Bosque arm (NB), and the main body (MR) of Waco Reservoir; August - November, 1968. Standing crop expressed as the total number of identifiable phytoplankton per milliliter.

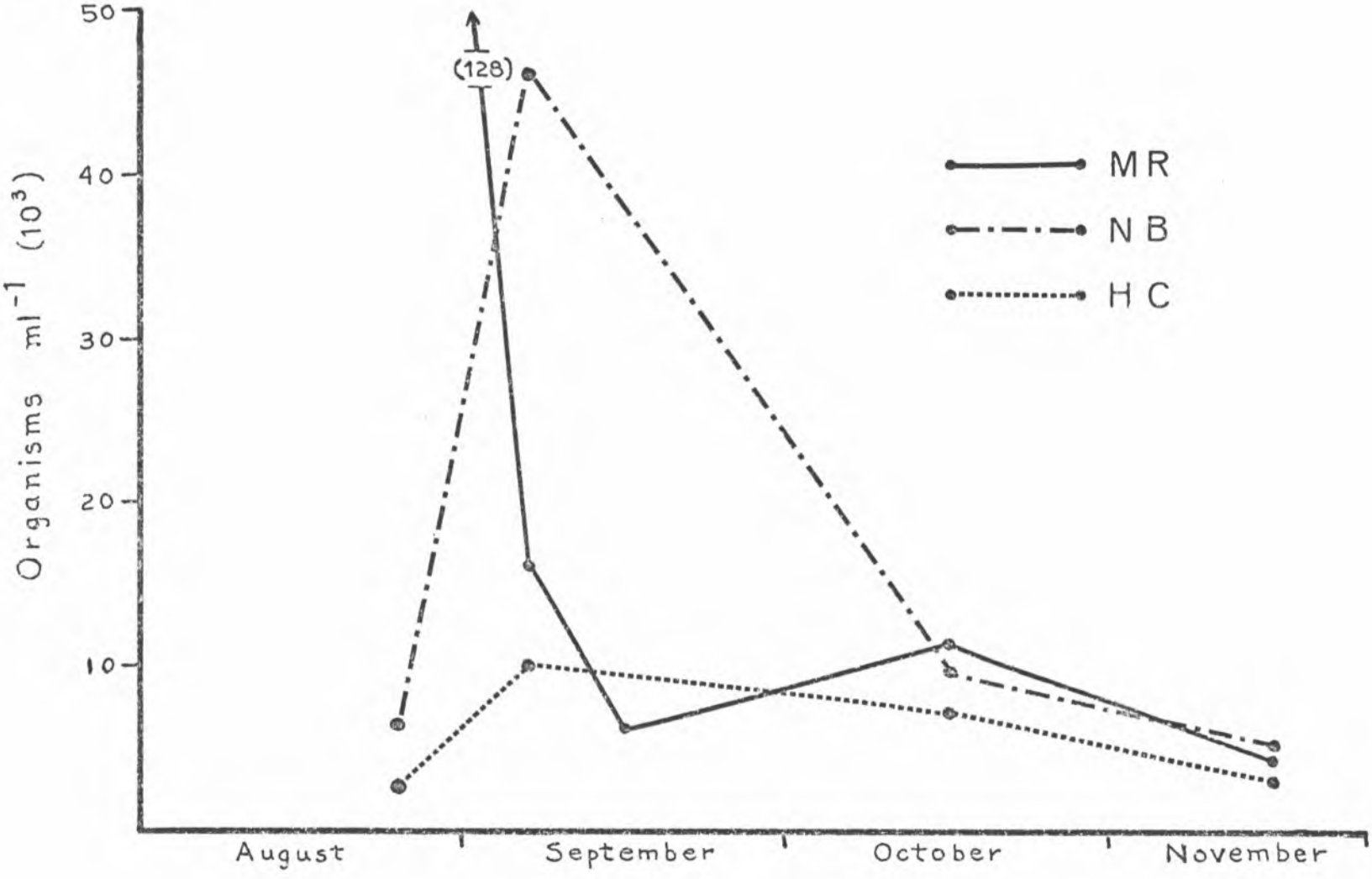

50
40
30
20
10
(128)
Organisms ml^{-1} (10^3)
MR
NB
HC
August
September
October
November

Table 6. Mean phytoplankton standing crop* (as total number of identifiable organisms per milliliter) and mean adjusted to eliminate bias effects of blooms at Waco Reservoir stations, August - November, 1968.

Station	Mean Total Organisms ml^{-1} (10^3) Unadjusted	Adjusted
Hog Creek Arm	5.72	5.72
North Bosque Arm	16.64	6.85
Main Body of Reservoir	32.93	9.24

* See Appendix I for phytoplankton genera and organisms present on each sampling date.

Table 7. Mean proportion of diatoms in the phytoplankton standing crop* and mean diatom proportion adjusted to eliminate bias effects of blooms, August - November, 1968.

Station	Mean Diatom Percentage of Average Phytoplankton Standing Crop	
	Unadjusted	Adjusted
Hog Creek Arm	69	69
North Bosque Arm	55	34
Main Body of Reservoir	27	17

*See Appendix I for phytoplankton genera and organisms present on each sampling date.

occurred during June and August, following tributary inflow peaks in May and July. The North Bosque arm had the highest average primary production on both surface and volume bases (Table 8). Primary production per square meter at HC was lower ($P = 0.05$) than at NB and MR, which were not significantly different. Production per cubic meter was significantly higher in the North Bosque arm. Hog Creek averaged more productive per unit volume than MR, but this difference was not statistically significant.

The photosynthetic efficiency of light utilization was consistently highest in the North Bosque arm, although that at MR was not significantly less. Photosynthetic efficiency at both NB and MR was significantly greater ($P = 0.05$) than that at HC.

Photosynthetic Response to Artificial Enrichment

Primary production response to artificial nutrient enrichment varied inversely with nutrient availability throughout the study. Nutrient addition produced positive photosynthetic responses in the reservoir arms only during periods of relatively low primary production. Carbon fixation in control samples exceeded that in experimentally enriched HC and NB samples during periods of high photosynthetic activity (Figs. 10 and 11). Experimental enrichment consistently stimulated production at MR, even during peaks of photosynthetic activity (Fig. 12). Primary production on both surface and volume bases exhibited an overall positive response to artificial enrichment (Table 9), although only MR production per cubic meter was increased significantly ($P = 0.01$).

Bioassay for Limiting Nutrient Factors

Average primary production responses to nutrient addition during

Table 8. Mean primary production and photosynthetic efficiency of light utilization at Waco Reservoir stations.

Station	Mean Primary Production (mg C m^{-2} day^{-1})	Observed Range (mg C m^{-2} day^{-1})	Mean Primary Production (mg C m^{-3} day^{-1})	Observed Range (mg C m^{-3} day^{-1})	Mean Photosynthetic Efficiency (%)	Observed Range (%)
Hog Creek Arm	578	176-1113	319	147-539	0.24	0.07-0.43
North Bosque Arm	1103	378-2076	583	196-1266	0.43	0.16-0.69
Main Lake	890	220-1449	269	71-397	0.38	0.07-0.70

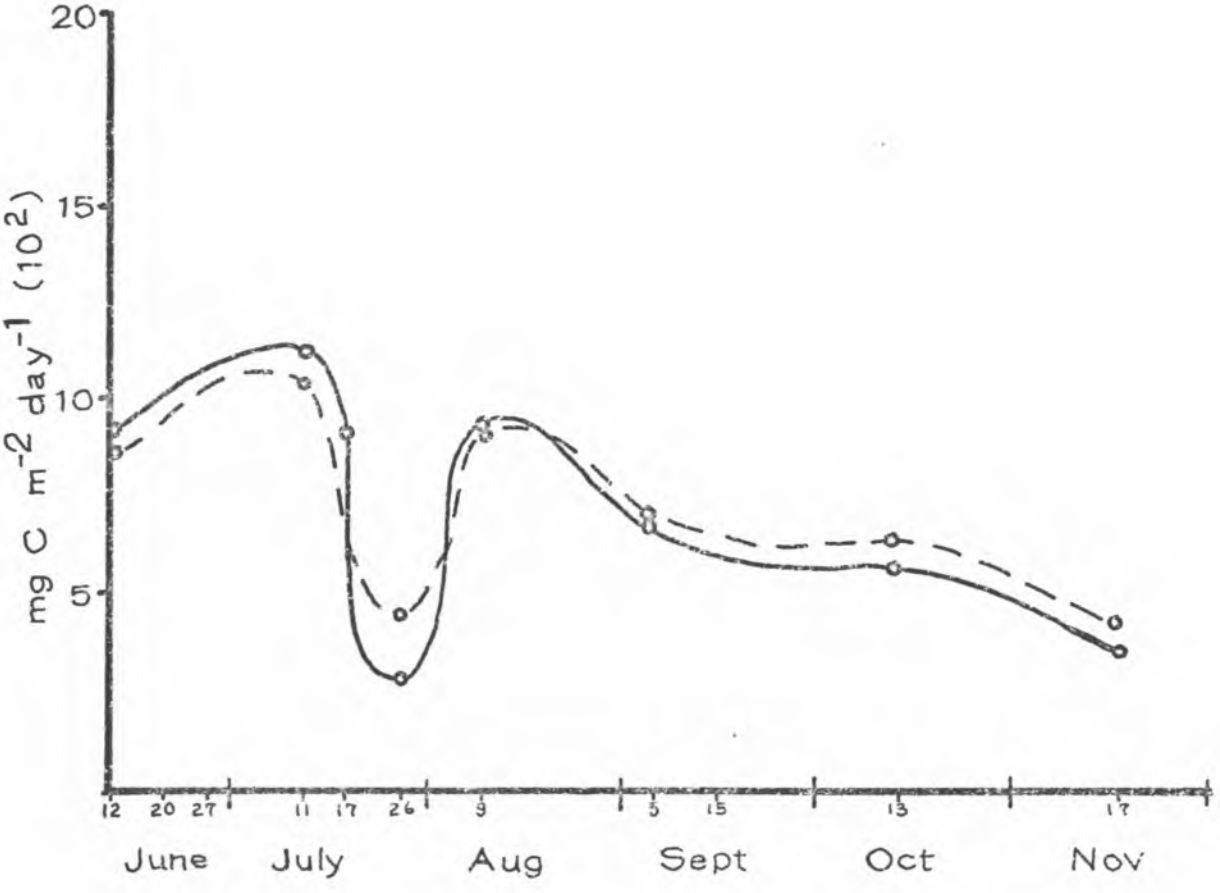
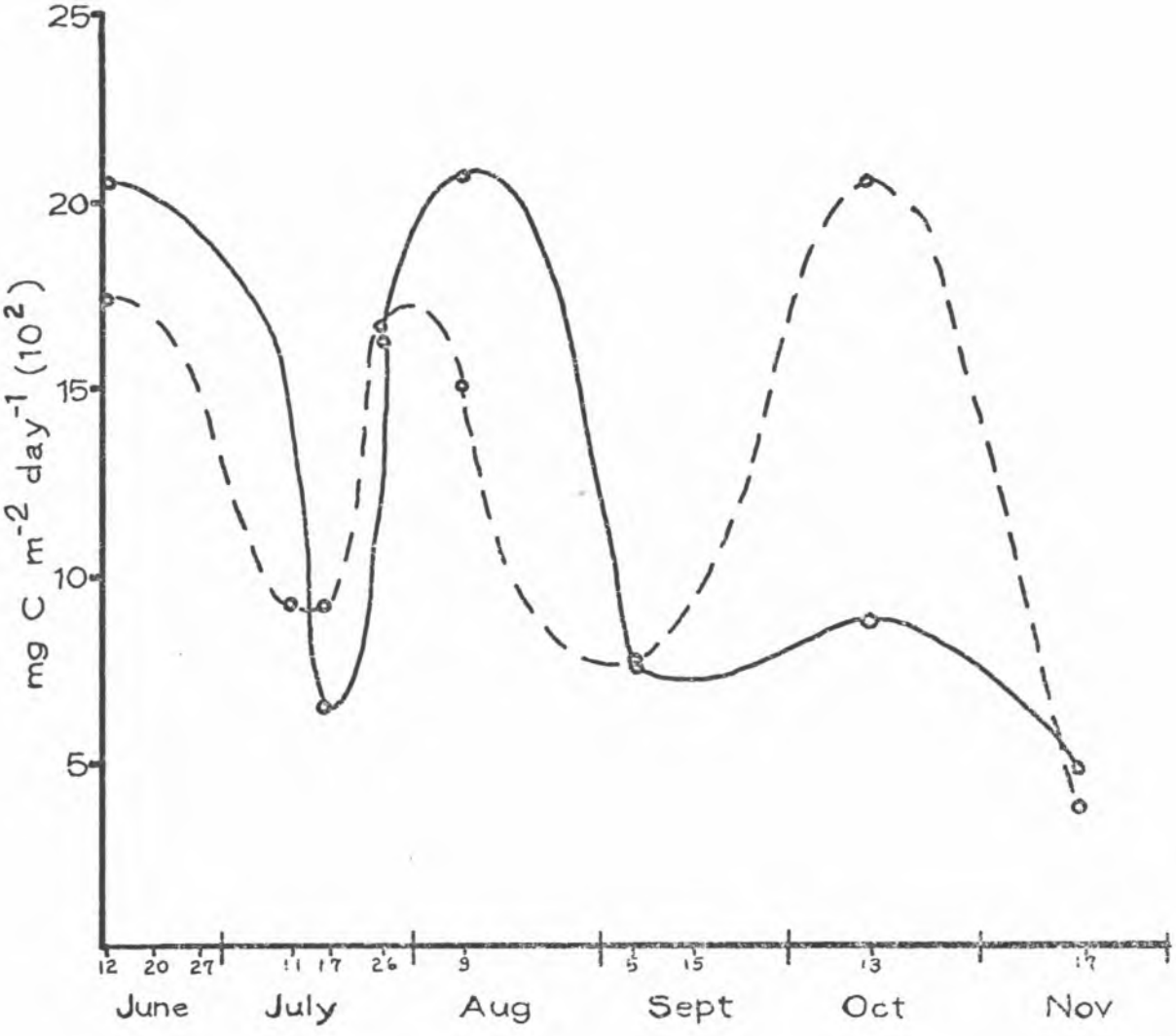


Figure 10. Phytoplankton production in control (solid line) and experimentally enriched (broken line) samples in the Hog Creek arm, Waco Reservoir.



Figrue 11. Phytoplankton production in control (solid line) and experimentally enriched (broken line) samples in the North Bosque arm, Waco, Reservoir.

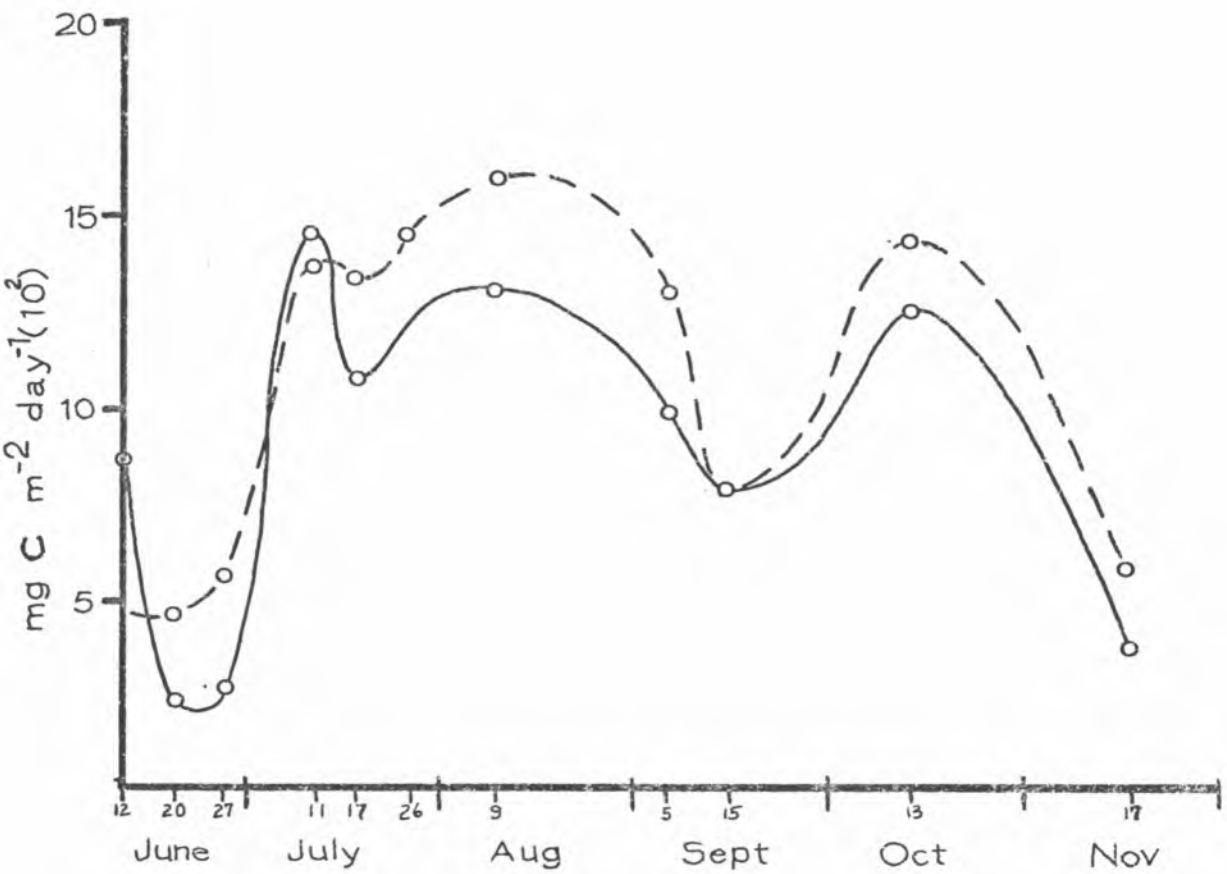


Figure 12. Phytoplankton production in control (solid line) and experimentally enriched (broken line) samples in the main body of Waco Reservoir.

Table 9. Mean effect of experimental nutrient enrichment on primary production at Waco Reservoir stations. Production values are expressed in terms of area and volume.

Station	g C m^{-2}			g C m^{-3}		
	Control	Enriched	Response to Enrichment	Control	Enriched	Response to Enrichment
Hog Creek	222	231	+4%	123	128	+4%
North Bosque	381	405	+6%	181	193	+7%
Main Lake	297	355	+20%	96	114	+19%

in situ ^{14}C bioassay experiments are given in Table 10. Of the nutrient additives tested, only silica consistently stimulated carbon fixation at all three stations. Chelated ferric iron addition produced slightly positive responses at all stations. Carbon fixation in Hog Creek samples was slightly stimulated by phosphate, nitrate, and EDTA addition. Other nutrient additions produced slightly negative effects on photosynthetic activity.

Factors Affecting Primary Production

Physical Factors

Light extinction by silt turbidity exerted the most significant physical limiting influence on phytoplankton production. Water temperature and solar radiation generally corresponded to primary production, but neither was significantly correlated with it (Tables 11, 12, and 13). Light inhibition of surface photosynthesis occurred at all stations, but more consistently at HC and MR (Fig. 13). Analysis of variance indicated that surface carbon fixation was significantly lower than at the 75% surface illumination depth at HC ($P = 0.01$) and MR ($P = 0.05$) Appendix F. Surface light inhibition corresponded more to light extinction than to solar radiation intensity. Surface light inhibition was inversely related to the light extinction coefficient (Fig. 14).

Turbidity limited primary production at all stations, but especially in the reservoir arms. Silt carried in by tributaries increased light extinction and thus, decreased primary production per unit surface area. Photosynthetic efficiency and production per square meter were severely limited by turbidity at HC. Turbidity did not significantly decrease production per cubic meter (Tables 11, 12, and 13). Inhibition of production per unit surface area was primarily a result of decreased photic depth.

Table 10. Mean response of phytoplankton growth to nutrient addition during *in situ* ^{14}C bioassay experiments at Waco Reservoir stations, August - September, 1968. Results expressed as percent increase (+ %) or decrease (- %) from controls.

Nutrient Added*	Percent Response		
	Hog Creek Arm	North Bosque Arm	Main Lake
PO_4	+12	-10	-9
NO_3	+6	-4	-12
Si	+490	+780	+1050
Fe-EDTA	+28	+10	+11
EDTA	+40	-8	-4
SO_4	-12	-15	-38
Micronutrient	-5	-27	-27
Complete	-38	-9	-2

*See Table 2 for reagents used as nutrient sources, and for micronutrient mixture components.

Table 11. Linear correlation coefficients (r) calculated for environmental variables affecting photosynthetic efficiency of light utilization, primary production, and turbidity in the Hog Creek Arm of Waco Reservoir.

Independent Variables	Dependent Variables			
	Photosynthetic Efficiency	Production m^{-2}	Production m^{-3}	Turbidity
Solar Radiation	-	+0.124	+0.296	-
Surface Water Temperature		+0.260	+0.324	-
Turbidity	-0.802*	-0.720	-0.314	-
pH	-0.740*	+0.898*	+0.445	-0.620
Alkalinity	+0.148	+0.382	+0.130	+0.189
Free CO_2	+0.991*	-0.792*	-0.667*	+0.738
Phosphate - Phosphorus	-0.693	-0.507	-0.084	+0.596
Silica	+0.999*	+0.935*	+0.998*	-0.414
Sulfate	-0.919*	-0.900*	-0.631	+0.925*
Specific Conductance	+0.288	-0.422	+0.171	-0.236
Phytoplankton Standing Crop	+0.944*	0.980*	+0.974*	-0.664
Diatom Standing Crop	+0.962*	+0.992*	+0.985*	-0.620

*Indicates statistical significance at the 0.05 level (P = 0.05).

Table 12. Linear correlation coefficients (r) for environmental variables Affecting photosynthetic efficiency of light utilization, primary production, and turbidity in the North Bosque Arm of Waco Reservoir.

Independent Variables	Dependent Variables: Photo-synthetic Efficiency	Production m^{-2}	Production m^{-3}	Turbidity
Solar Radiation	-	+0.523	+0.715*	-
Surface Water Temperature		+0.451	+0.428	-
Turbidity	-0.457	-0.169	+0.348	-
pH	+0.736*	+0.716*	+0.373	+0.344
Alkalinity	-0.353	-0.252	+0.273	+0.859*
Free CO_2	+0.815*	-0.593	-0.402	+0.031
Phosphate-phosphorus	-0.264	-0.004	+0.267	+0.426
Silica	+0.866	+0.686	+0.999*	+0.344
Sulfate	-0.862*	-0.813*	-0.355	+0.347
Specific Conductance	-0.687	-0.814	-0.723	-0.125
Phytoplankton Standing Crop	+0.589	+0.333	+0.926	+0.694
Diatom Standing Crop	+0.584	+0.326	+0.924	+0.699

*Indicates statistical significance at the 0.05 level (P = 0.05).

Table 13. Linear correlation coefficients (r) for environmental variables affecting photosynthetic efficiency of light utilization, primary production, and turbidity in the main body of Waco Reservoir.

Independent Variables	Dependent Variables			
	Photo-synthetic Efficiency	Production m^{-2}	Production m^{-3}	Turbidity
Solar Radiation	-	+0.162	+0.126	-
Surface Water Temperature		+0.375	+0.311	-
Turbidity	+0.043	-0.343	-0.030	-
pH	+0.567	+0.774*	+0.746*	-0.397
Alkalinity	-0.126	-0.231	-0.040	+0.320
Free CO_2	-0.248	-0.829*	-0.815*	+0.757*
Phosphate-phosphorus	-0.163	-0.401	-0.092	+0.415
Silica	+0.864*	+0.669	+0.428	+0.118
Sulfate	+0.313	+0.531	+0.622	-0.405
Specific Conductance	+0.201	-0.184	-0.076	+0.470
Phytoplankton Standing Crop	+0.856	+0.710	+0.698	-0.552
Diatom Standing Crop	+0.823	+0.616	+0.590	-0.701

*Indicates statistical significance at the 0.05 level (P = 0.05).

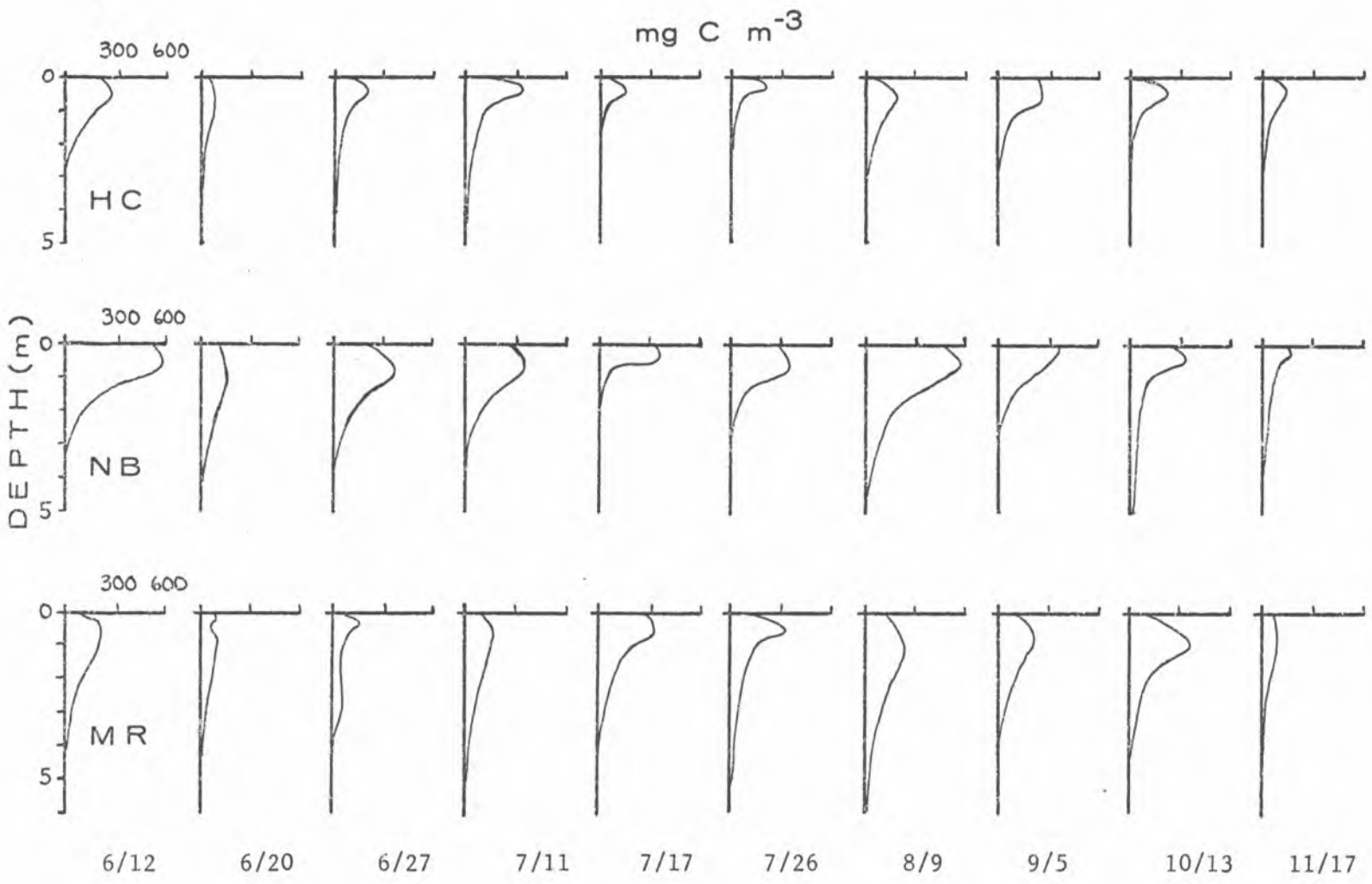


Figure 13. Net phytoplankton production depth profiles for Waco Reservoir sampling stations from June - November, 1968.

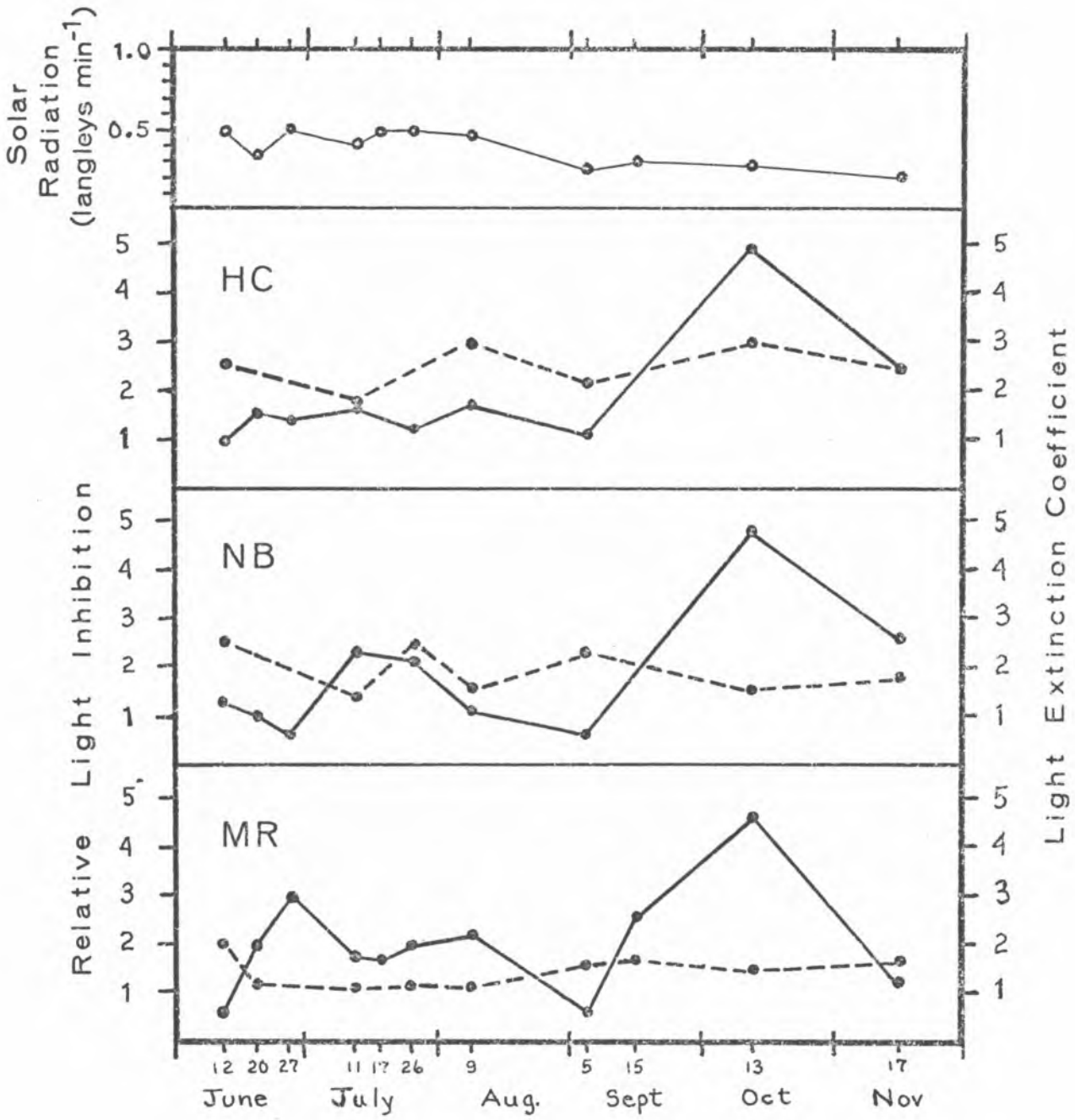


Figure 14. Relationship of the relative light inhibition of photosynthesis to photosynthetic solar radiation (o—o) and light extinction (•---•). Relative surface light inhibition (•—•) is expressed as the ratio of carbon fixation at 75% surface light intensity to that at the surface.

Turbidity was positively correlated with free CO_2, alkalinity, and PO_4-P at all stations, and with silica at NB and MR. It was also positively correlated with sulfate at HC and NB (Tables 10 and 11). Partial correlation analysis[10] indicated that the limiting influence of increased turbidity often obscured primary production stimulation by nutrient inflow.

Chemical Factors

Free CO_2 concentration was positively correlated with photosynthetic efficiency ($P = 0.05$) and negatively correlated with primary production on surface and volume bases ($P = 0.05$). Positive correlations were found between pH and photosynthetic efficiency, production per square meter, and production per cubic meter at each station. Primary production on surface or volume bases was not significantly related to alkalinity or specific conductance (Tables 11, 12, and 13).

Photosynthetic efficiency at HC was negatively related to increased concentrations of PO_4-P ($P = 0.05$) and SO_4 ($P = 0.05$). Increased SO_4 concentration was negatively correlated with photosynthetic efficiency at NB ($P = 0.05$), but the two were positively correlated ($P = 0.05$) at MR (Tables 12 and 13).

In situ Limiting Nutrient Bioassay and Phosphate Enrichment Experiments

Limiting nutrient bioassay results indicated that of the nutrient additives tested, only silica stimulated primary production in Waco Reservoir. Addition of other macro- and micronutrients had no consistent effect on ^{14}C fixation (Table 10).

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[10]Refer to Appendix H for partial correlation analysis summary.

Phosphate additions during nutrient bioassay produced negative responses at each station, although enrichment experiments in isolated water columns suggested that phosphate occasionally limited primary production.

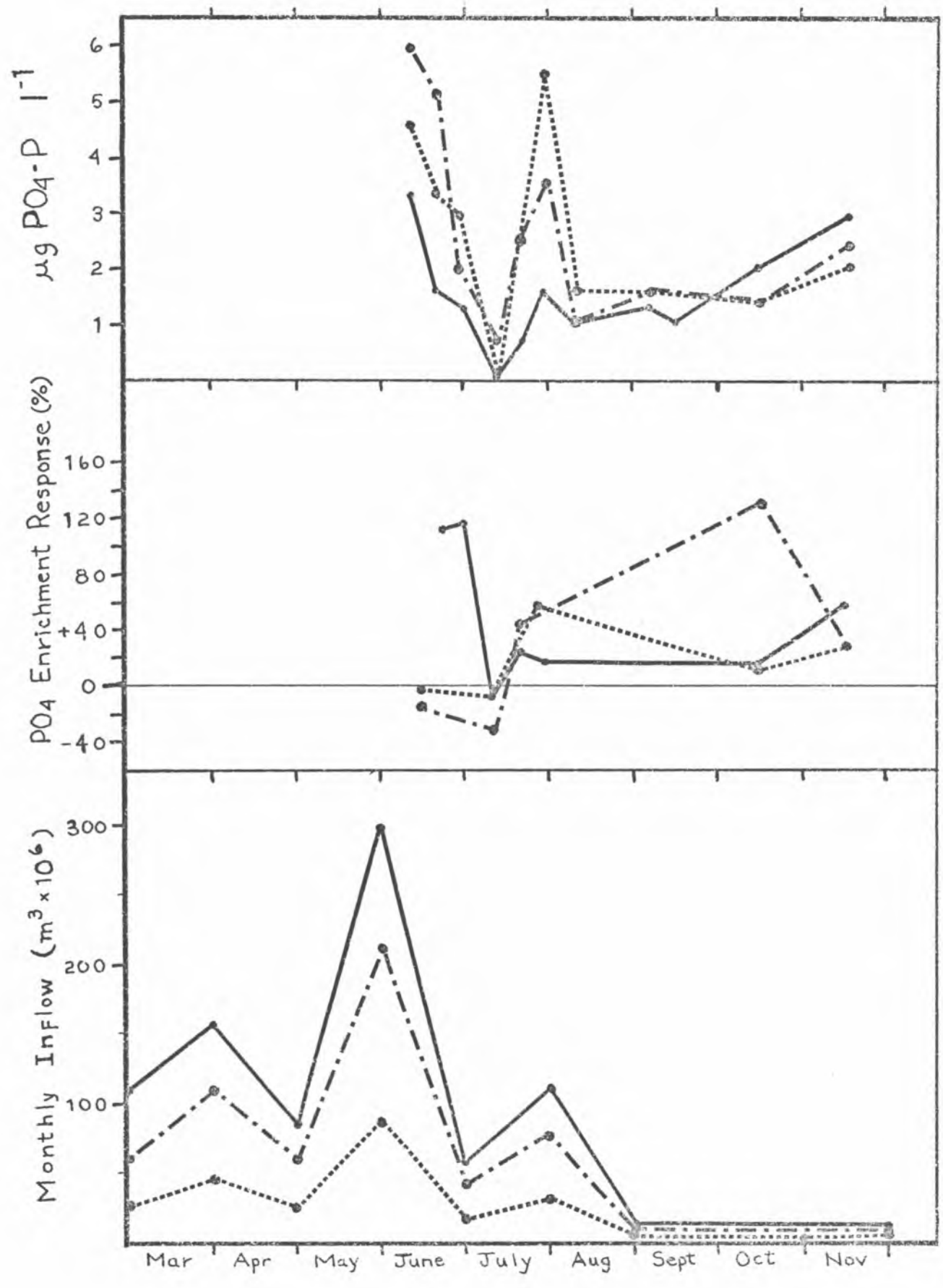
Primary production was consistently increased at MR by phosphate enrichment, but was stimulated at HC and NB only during intervals of low photosynthetic activity (Figs. 10, 11, and 12). Phosphate-phosphorus concentrations varied directly with tributary inflow (Fig. 15) and inversely with photosynthetic activity (Tables 11, 12, and 13). Phosphate was not limiting to primary production in the reservoir arms until mid-July, although phosphate concentrations accumulated by spring surface runoff were depleted by late June. Experimental phosphate addition stimulated primary production at HC and NB from August through November. This response corresponded to reduced tributary inflow and decreased phosphate concentrations (Fig. 15).

Regular positive responses to in situ phosphate enrichment indicated that phosphate was consistently limiting to primary production at MR. The only negative response to phosphate addition at MR occurred when an unknown factor became limiting on 11 July (Fig. 12). High rates of photosynthetic activity depleted PO_4-P and free CO_2 concentrations, but additions of phosphate failed to stimulate production.

Biological Factors

Diatoms were the dominant primary producers from August through November, 1968. Since diatom standing crop and silica concentrations were highly correlated with each other, and with primary production (Tables 11, 12, and 13), partial correlation coefficients were calculated to distinguish the influence of the nutrient from that of diatom density. Results

Figure 15. Comparison of photosynthetic response to *in situ* PO_4 enrichment, analytically determined PO_4-P concentrations, and tributary inflow at Hog Creek (·····), North Bosque (— — —), and Main Reservoir (——) stations.


µg PO4-P l⁻¹
6
5
4
3
2
1
PO4 Enrichment Response (%)
160
120
80
+40
0
-40
Monthly Inflow (m³ x 10⁶)
300
200
100
Mar
Apr
May
June
July
Aug
Sept
Oct
Nov

indicated that the presence of large diatom populations decreased photosynthetic efficiency at HC and NB. Positive linear correlations between diatoms and photosynthetic efficiency are actually due entirely to silica concentrations (Appendix H). Increased diatom numbers had significant, but opposite, effects on primary production per unit surface area in HC and NB. Production per square meter was decreased ($P = 0.05$) by increased diatom density at NB, but was positively correlated at HC. Diatom numbers were not significantly correlated with either photosynthetic efficiency or production per square meter at MR (Appendix H).

DISCUSSION

Factors Affecting Primary Production in Waco Reservoir

Phytoplankton production was greater in the reservoir arms near nutrient inflow sources, than in the main body of Waco Reservoir. Higher production per unit volume at HC and NB than at MR (Table 8) indicated that inflowing nutrients enriched Hog Creek and North Bosque arms. Goldman (1960b) and Goldman and Carter (1965) reported higher production near areas of tributary inflow. Photosynthetic nutrient utilization in HC and NB reduced nutrient availability at MR. The MR carbon fixation response to experimental phosphate enrichment (Table 9) indicated slight nutrient limitation of MR production. Positive correlations of MR photosynthetic efficiency with PO_4-P and SO_4 also suggested nutrient limitation. However, continuous wind mixing of water at MR resulted in rapid nutrient turnover, which minimized nutrient limitation.

Alteration of nutrient factors had little effect on primary production in the reservoir arms. Experimental phosphate enrichment produced negligible stimulation of production at HC and NB (Table 9). Goldman and Wetzel (1963) reported little stimulation of carbon fixation resulting from nutrient addition to samples from a shallow, eutrophic, California lake. Negative relationships between primary production and nutrient concentrations (PO_4-P, SO_4) in the reservoir arms (Appendix H) were probably not due to nutrient inhibition of production, but to phytoplankton standing crop dilution by inflowing waters (Findenegg 1965). Nutrient

concentrations increased during periods of increased tributary inflow (Fig. 15).

Free CO_2 was negatively correlated and pH was positively correlated with primary production at all stations (Tables 11, 12, and 13). Photosynthetic efficiency and primary production rates were directly correlated with pH. Free CO_2 concentrations were inversely related to carbon fixation rates and directly related to photosynthetic efficiency. Although photosynthetic activity significantly reduced free CO_2 concentrations, phytoplankton production was not limited by CO_2 shortage because of its availability as bicarbonate alkalinity (Ruttner 1963).

Silica addition produced considerable increases in carbon fixation rates during in situ bioassay experiments, but the nutrient is of doubtful significance as a limiting factor. The stimulating effect of silica addition on primary production corresponded to diatom dominance of the phytoplankton standing crop (Table 7). Large diatom populations were maintained in all reservoir areas by relatively high silica concentrations (Table 5). The lowest silica concentration recorded (5.8 mg l^{-1}) during this study was in great excess of that (0.5 mg l^{-1}) reported to limit freshwater diatom production (Pearsall 1932). Positive correlations of silica concentration with diatom standing crop and primary production in Waco Reservoir (Tables 11, 12, and 13) indicated that diatom growth was not limited by low silica availability, but that it occurred opportunistically in high nutrient concentrations. Dugdale (1967) reported that diatoms are generally dominant in nutrient-rich marine areas, probably because their high growth rates correspond to high rates of nutrient uptake. Lund (1964) and Goldman et al. (1968) found negative relationships between silica concentration and diatom fluctuations in waters

of lower silica content.

Diatom dominance and blooms were recorded for many central Texas impoundments. Palmer (1964) reported that blooms of Melosira had occurred in Lakes Amarillo, Bridgeport, Caddo, Dallas, Eagle, Waco, and Worth in Texas. Increases in phosphate availability, when silicates were present from colloidal clay of the surrounding drainage basin, often produced Melosira or Synedra blooms. Synedra was the predominant bloom organism in Waco Reservoir although Melosira was present. Melosira and Stephanodiscus, both abundant in the reservoir, are widely recognized as indicators of eutrophic conditions (Rawson 1956). Lackey (1945) designated "bloom" conditions to occur when more than 500 individuals of a given phytoplankton species were present per milliliter of water. Waco Reservoir waters are in continuous "bloom" conditions by this criterion.

Light extinction was the most important limiting influence on primary production. Silt from tributary inflow, particulate matter suspended by water mixing, and large phytoplankton standing crops combined to limit light penetration and thus restrict the thickness of the photic layer. Verduin (1954) considered turbidity to limit primary production in western Lake Erie when the photic depth was reduced to less than 3.5 meters. Plant nutrient inflow was often accompanied by increased inorganic turbidity, which superimposed a light extinction gradient on the nutrient concentration gradient. The inhibiting influence of turbidity was most apparent at HC, which was significantly more turbid than NB or ML. Increased light extinction often masked primary production stimulation by inflowing nutrients at HC. The high turbidity of the Hog Creek arm was due primarily to drainage from the South Bosque River, the principal silt contributor to Waco Reservoir. Although HC had greater nutrient enrichment and was more productive per unit volume than MR, the main

body was more productive per unit surface area due to its greater photic depth. High silt turbidity was responsible for lower production per unit surface area and lower photosynthetic efficiency at HC than in less turbid area of Waco Reservoir. Goldman and Wetzel (1963) found that when high turbidity accompanied nutrient inflow, primary production was frequently higher in more transparent areas downstream from the point of inflow. Ryther (1956) concluded that light always limits natural photoplankton production in some way, and limiting effects of nutrient deficiencies are merely additive to the light factor.

Organic turbidity, produced by diatom blooms, decreased light penetration and ultimately reduced photosynthetic production at NB. Diatom density was positively correlated with turbidity only at NB (Table 12). Stimulated diatom growth increased primary production and photosynthetic efficiency until the diatom density became so great that self-regulatory effects were produced. Shading increased with the diatom population size, and reduced photosynthetic efficiency. Talling (1960) and Wetzel (1965a) found that self-regulation of photosynthesis occurred primarily by shading and photic zone reduction. Diatom growth affected primary production differently at HC and NB. Surface area primary production was negatively correlated with diatom density at NB and positively correlated at HC, although diatom numbers reduced photosynthetic efficiency at both. These contrasting effects were related to water transparency differences at HC and NB. Photosynthetic efficiency reduction by diatom shading was negligible in the turbid Hog Creek arm compared to the production increase caused by large diatom populations. In the more transparent waters of NB, diatom shading significantly inhibited production per unit surface area by decreasing the light energy available for photosynthesis in the deeper

regions of the photic column.

Although inorganic turbidity and high diatom density decreased production per unit surface area, these light extinction factors also reduced light inhibition of photosynthesis. Surface light inhibition has often been observed during in situ primary production measurements (Edmondson 1956; Talling 1957; Goldman 1960; Goldman, Mason, and Wood 1963; Goldman and Wetzel 1963; Lind 1966; and Goldman et al. 1968) and was frequently noted in Waco Reservoir experiments. The inhibitive effects of light intensities in excess of 0.2 langley min^{-1} (langley = 1 g-cal cm^{-2}) on photosynthesis have been detected in natural phytoplankton populations (Talling 1961). Elster (1965) reported that surface inhibition was often widely independent of absolute light intensity and depended more on surface radiation quality and the physiological status of the producers. Photosynthetically active solar radiation exceeded 0.2 langley min^{-1} throughout this study, ranging from 0.52 langley min^{-1} in late June to 0.21 langley min^{-1} in mid-November. The degree of light inhibition at Waco Reservoir stations was more dependent on water turbidity than light intensity (Fig. 14). Turbidity produced by high plankton density reduced surface light inhibition at NB. No surface inhibition occurred at NB on 27 June and 9 August, although photosynthetic activity in surface layers was inhibited at HC and MR (Fig. 13). These dates correspond to high primary production periods at NB (Fig. 11).

Continuous wind mixing of water in the reservoir main body produced feedback effects on production. Phytoplankton production is a function of incident radiation, mixing depth, respiration, and water transparency; with transparency being a function of the producer density (Murphy 1962).

Mixing prevented stable thermal stratification and no hypolimnetic isolation of plant nutrients occurred. Well-oxygenated water promoted bacterial decomposition of organic matter and rapid nutrient turnover, but may have caused some nutrients to be precipitated (Einsele 1938). Continuous water circulation provided a constant nutrient supply available for photosynthesis, and thus maintained high primary production rates. However, continuous mixing also increased light extinction by maintaining particulate matter in suspension and stimulating phytoplankton production. Light extinction often severely limited primary production by restricting the photic zone, but also decreased surface light inhibition.

Stratified aerobic waters often function as nutrient traps when nutrients are precipitated and lost to the sediments (Einsele 1938; Mortimer 1941; and Hayes and Phillips 1958). Constant water circulation maintains suspended nutrients associated with precipitates, silt particles, and decaying organic material, and thus minimizes nutrient loss to the sediments. Continued water movement produces steep nutrient diffusion gradients at the sediment-water interface, and thus maintains high rates of nutrient influx from sediments to water. The dynamic equilibrium between sediment and water nutrient reservoirs (Mortimer 1941; Hayes and Phillips 1958; and Olsen 1964) provides a continuous inflow of available nutrients in a mixing system.

Dugdale (1967) considered primary production limitation in the sea to be primarily due to inadequate light and insufficient nutrient availability. He distinguished between an upper photic zone region with adequate light but limiting nutrient concentrations, and a lower region where light is less available than nutrients. If this is also the case

in freshwater systems, shallow polymictic environments, such as Waco Reservoir, may provide near optimal conditions for primary production by the continuous circulation of producers and nutrients through a light intensity gradient. Circulation of water through the entire reservoir volume certainly carries a portion of the productive phytoplankton community out of the photic zone. Mixing depth can limit primary production if it causes the producers to spend too large a fraction of their lives at depths receiving insufficient light (Sverdrup 1953).

Waco Reservoir primary production values were probably underestimated, especially those for the main body of the impoundment where the most intense mixing occurred. Phytoplankton enclosed in a bottle at one particular depth lose advantages of continuous circulation (Goldman and Wetzel 1963). This fact may be of considerable importance in constantly mixing systems. Phytoplankton cells store energy during brief exposures to light and utilize it later when at lower light intensities (Antia et al. 1963). The photosynthetic activity of cells brought to the surface is probably not significantly reduced by light injury as recovery in rapidly achieved (Goldman et al. 1963). Ryther and Menzel (1959) and Williams and Murdoch (1966) also reported the ability of phytoplankton exposed to tubulent mixing to adapt to changing illumination. Further investigation of the physiological effects of continuously varying light intensities and periodic light absences on the photosynthetic ability of natural phytoplankton communities is necessary before the ecological implications of mixing aquatic systems can be interpreted adequately.

Trophic Status of the Waco Reservoir Community

The trophic level of a body of water is a function of its fertility (Ohle 1956). The best available assessment of aquatic fertility is an

estimate of the production of the producer community (Goldman and Wetzel 1963). Comparison of Waco Reservoir fertility with other aquatic systems is difficult because most investigators have reported production rates only in terms of surface area. Production per unit surface area often does not reflect the trophic status of water bodies because of differences in productive zone thickness. Production per unit volume of photic zone is a more informative index of aquatic fertility (Ruttner 1963). To facilitate comparison, several daily surface production values reported in the literature were converted to a volume basis by dividing by the photic depth or mean depth of the system. If given, photic depth was used for the conversion.

Comparison of Waco Reservoir primary production with other bodies of water (Table 14) shows the impoundment to be in an advanced stage of eutrophication and among the most productive freshwater systems yet studied. Phytoplankton production at all stations exceeds that of Clear Lake (Goldman and Wetzel 1963) and approaches that of Borax Lake (Wetzel 1964). North Bosque production per unit volume exceeds that of an Indiana lake polluted by nutrient enrichment (Wetzel 1965a). Few annual primary production estimates have been made for freshwater systems (Table 15), although Strickland (1960) and Gilmartin (1964) listed several for marine environments. Waco Reservoir is less productive than the British Columbia fjord studied by Gilmartin (1964).

The Fate of Waco Reservoir

Most impoundments are inherently exposed to more intense forces of eutrophication than the typical natural lake due to their position as bariers to natural surface drainage. Reservoirs act as settling basins for sediment and as traps for plant nutrients and organic matter. Large

Table 14. Comparison of mean net primary production values for several lakes to those for Waco Reservoir, Texas. Bodies of water arranged in order of increasing productivity.

Body of Water	Mean Net Primary Production: mg C m^{-2} day^{-1}	Mean Net Primary Production: mg C m^{-3} day^{-1}	Method of Estimation	Remarks
Lake Vanda, Antarctica[a]	14.	0.24	^{14}C	
Lake S-7, Canadian arctic[b]	9	1.5	^{14}C	Oligotrophic lakes, characterized
Lake Tahoe, California-Nevada[c]	28-133	1.65	^{14}C	by transparent water and deep
Lake Bonney, Antarctica[a]	31	3.1*	^{14}C	euphotic zones.
Brooks Lake, Alaska[d]	158	3.5	^{14}C	
Bare Lake, Alaska:[e]				
Pre-fertilization	100	25	O_2	Fertility increased by artificial
Post-fertilization	-	150-200	O_2	fertilization.
Crooked Lake, Indiana[f]	469.2,358.7	31*,24*	^{14}C	Deep unproductive calcareous lake. Values for 1963 and 1964 respective.
Lake S-1, Canadian arctic[b]	99	50	^{14}C	Shallow tundra pond.
Imikpuk Lake, Alaska[g]	120	58	O_2	Shallow lake.
Martin Lake, Indiana[f]	561	56**	^{14}C	Deep unproductive calcareous lake.
Smith Hole, Indiana[f]	194	97*	^{14}C	Small dystrophic lake.
Clear Lake, California[h]	438	146	^{14}C	Shallow, wind-mixed, eutrophic lake.

Table 14. (continued)

Body of Water	Mean Net Primary Production: mg C m^{-2} day^{-1}	Mean Net Primary Production: mg C m^{-3} day^{-1}	Method of Estimation	Remarks
Waco Reservoir, main body[i]	890	269	^{14}C	
Waco Reservoir, Hog Creek arm[i]	578	319	^{14}C	
Borax Lake, California[j]	249	356**	^{14}C	Very shallow, turbid, eutrophic.
Waco Reservoir, mean value[i]	857	390	^{14}C	
Sylvan Lake, Indiana[f]	1564	489**	^{14}C	Polluted by nutrient enrichment.
Waco Reservoir, North Bosque arm[i]	1103	583	^{14}C	
Lake Erken, Sweden[k]	40-2205	730	^{14}C	Eutrophic

*Production per square meter divided by literature value for photic depth to estimate phytoplankton production per unit volume

**Production per square meter divided by literature value for mean depth to estimate phytoplankton production per unit volume

Sources of data: (a) Goldman, Mason, and Hobbie, 1967; (b) Frey and Stahl, 1958; (c) Goldman and Carter, 1965; (d) Goldman, 1960b; (e) Nelson and Edmondson, 1955; (f) Wetzel, 1965a; (g) Comita and Edmondson, 1953 from Frey and Stahl, 1958; (h) Goldman and Wetzel, 1963; (i) this study; (j) Wetzel, 1964; (k) Rodhe, 1958 from Saunders, 1964.

Table 15. Comparison of annual net primary production of several aquatic systems with that of Waco Reservoir, Texas.

Body of Water	Annual Net Primary Production ($g\ C\ m^{-2}\ yr^{-1}$)	Source of Data
Lunzer Untersee, Austria	30	Steeman Nielsen (1959)
Lake Tahoe, Nevada-California	36	Goldman and Carter (1965)
Clear Lake, California	160	Goldman and Wetzel (1963)
Waco Reservoir, Texas:		
North Bosque arm	381	
Main Body	297	
Hog Creek arm	222	
Mean value for reservoir	300	
British Columbia fjord	455	Gilmartin (1964)

nutrient accumulations may become isolated in the hypolimnion of thermally stratified reservoirs and remain unavailable for primary production during most of the summer growing season. Thermal stratification in shallow bodies of water is generally prevented by wind, and nutrients released by bacterial decomposition of organic detritus are rapidly recirculated for continuous plant production (Kerekes and Nursall 1965; Fruh 1967). High rates of photosynthetic production are maintained in Waco Reservoir by initial nutrient inflow from the drainage basin and by rapid nutrient regeneration. The influence of mixing on primary production becomes more important as the reservoir basin is filled. Turbidity is less depressing to production if the mixing depth is shallow (Murphy 1962). Eutrophication of the reservoir is accelerated not only by sedimentation, but also by continuously higher rates of organic matter production. Ageing of Waco Reservoir should increase rapidly with time as the basin becomes shallower and phytoplankters spend an increasingly larger fraction of their lives in the productive zone.

SUMMARY

Waco Reservoir was found to be a very productive, polymictic impoundment undergoing accelerated eutrophication. Mean net phytoplankton production was 269 mg C m^{-3} day^{-1} (890 mg C m^{-2} day^{-1}) in the reservoir main body, 319 mg C m^{-3} day^{-1} (578 g C m^{-2} day^{-1}) in the Hog Creek arm, and 583 mg C m^{-3} day^{-1} (1103 mg C m^{-2} day^{-1}) in the North Bosque arm. Average production for the impoundment was 390 mg C m^{-3} day^{-1} (857 mg C m^{-2} day^{-1}). Annual net phytoplankton production was 297 g C m^{-2} yr^{-1} in the reservoir main body, 222 g C m^{-2} yr^{-1} in Hog Creek, 381 g C m^{-2} yr^{-1} in the North Bosque arm, and 300 g C m^{-2} yr^{-1} average for the reservoir.

Nutrient inflow from the drainage basin increased primary production in tributary arms. The stimulating effect of nutrient inflow was often masked by accompanying inorganic turbidity. Phytoplankton production in the reservoir arms decreased nutrient availability for photosynthesis in the reservoir main body. Diatoms dominated the phytoplankton standing crop and bloomed in response to increased nutrient availability. Silica and phosphate stimulated diatom growth, but were not serious limiting factors. Light extinction by organic and inorganic turbidity was the most serious limiting factor to phytoplankton production.

Continuous wind-mixing of the reservoir accelerates its eutrophication. Rapid nutrient recirculation maintains nutrient availability for phytoplankton production throughout the growing season. Continuous circulation of phytoplankton and nutrients through a light intensity gradient may

provide near optimal conditions for phytoplankton production in aquatic systems. If so, Waco Reservoir will age more rapidly with time as the basin shallows and phytoplankters spend an increasingly larger fraction of their lives in the productive zone.

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APPENDIX A

Construction of Incubation Rack for *In Situ* ^{14}C Primary Production Measurements

The incubation rack used for ^{14}C sample bottle suspension was constructed of two 3 m lengths of 1.2 cm aluminum conduit suspended vertically on a polypropylene rope with 1.5 m lengths of conduit clamped horizontally to form cross-pieces. The upper end of the vertical support was clamped to the middle of a 3 m length of 2.5 cm conduit supported at each end by styrofoam floats (Fig. 4). "Jumbo" clamp holders[11] were used to secure horizontal supports to vertical supports thus allowing horizontal support vertical adjustment to depths corresponding to illumination levels. Ten small eye-bolts, to which sample bottles were snapped, were mounted on each horizontal cross-piece. Horizontal supports were so adjusted that bottles hung vertically with their midpoint at the appropriate illumination level depth. Sample bottles were spaced horizontally and cross-pieces arranged radially on the vertical axis to avoid sample shading. The apparatus was portable, easily disassembled, and stable even in relatively rough water.

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[11]Fisher Scientific Company

APPENDIX B

Construction of Polyethylene Cylinders and Uniform Nutrient Addition to Isolated Water Columns

Cylinders were made by taping 0.1 mm thick. 2.3 m width sheets of transparent polyethylene length-wise to form large open-ended plastic tubes 0.7 m in diameter and 5.5 m in length. Transparent polyethylene tape (5 cm width) was used to seal the cylinders. Wire hoops were taped at 1 m intervals to the outside of a cylinder for external support. Supporting hoops were made by bending 2.2 m lengths of 9-guage steel wire into circles and then forcing the ends into 10 cm lengths of 0.5 cm inner diameter plastic tubing. Top and bottom supporting hoops were made similarly from 4 cm by 2.3 m transparent plexiglas strips. The ends of these strips were joined with metal bolts. These supports were then secured to the polyethylene cylinder by folding the upper and lower ends of the plastic sheet back over the plexiglas hoops and taping. A completed cylinder is shown in Figure 5. In the water, the polyethylene cylinders were bouyed by attachment to 3 m lengths of 1.2 cm aluminum conduit supported at each end by styrofoam floats and anchored as shown in Figure 5. A kilogram weight was wired to the lower plexiglas ring to insure full vertical extension of the cylinder.

Isolated water column enrichment was accomplished by drawing nutrient solution into 1 cm inner diameter transparent plastic tubing until 5 m of the tubing was filled. The desired nutrient concentration for the water column was adjusted to the volume necessary to fill the tubing. The lower end of the tubing was then plugged with a counterweighted rubber stopper and

lowered slowly into the isolated water column until the level of solution within the tubing and that of the water were equal. Three minutes were allowed for the nutrient solution temperature to equilibrate to that of the surrounding water. The rubber stopper was dislodged by sharply jerking the upper end of the tubing. The tubing was slowly withdrawn from the water leaving the nutrient solution distributed throughout the water column. Nutrients were added on the evening preceding each sampling date. The 12 to 14 hour interval between nutrient addition and sampling time allowed added nutrients to be mixed through the isolated water column.

APPENDIX C

Calculation of ^{14}C Method Experimental Error

This method involves calculation of net carbon fixation and variance of net carbon fixation within each depth layer of the euphotic zone, followed by calculation of the carbon fixation of the entire euphotic water column and the integral variance. Data used in experimental error estimation is presented in Table 16. Net carbon fixation within each layer is equal to light fixation minus dark fixation multiplied by the thickness of the layer. Variance of net carbon fixation in samples from the same depth is:

$$S^2_{(net)} = S^2_{(light)} + S^2_{(dark)}$$

Since only a single dark bottle was incubated at each depth, the variance of dark fixation between depths was considered to approximate that in samples at the same depth and was substituted as $S^2_{(dark)}$ in the equation above. Variance of net carbon fixation within a layer is:

$$S^2_i = S^2_{(net)}\ t^2_i$$

where S^2_i = variance of net carbon fixation in the i th layer, $S^2_{(net)}$ = variance of the measured net uptake, and t_i - thickness of the i th layer. Integral net carbon fixation is the sum of net carbon fixation in all layers. Variance of integral net carbon fixation is the sum of the variance of all layers; i.e.,

$$S^2_{(integral)} = S^2_i$$

Table 16. Data used in estimation of experimental error of ^{14}C primary productivity measurements in Lake Waco reservoir; September 15, 1968.

Depth (m)	Thickness of layer (m)	Mean net counts $sec^{-1}m^{-3}$	Variance of net counts $sec^{-1}m^{-3}$
0	0.10	3.63	0.02
0.20	0.18	15.99	0.41
0.35	0.15	16.51	0.05
0.50	1.32	194.39	119.01
3.0	1.25	38.18	31.69

APPENDIX D

Calculation of Carbon Assimilation

$$P = \frac{r}{R} \times C \times f$$

Where: P = production (mg C m^{-3}).

r = uptake of radioactive carbon (counts min^{-1}).

R = total amount of radioactive carbon available for uptake (counts min^{-1}).

C = total amount of stable inorganic carbon available for uptake (mg C m^{-3}).

f = correction factor (1.06) for isotope effect.

The above are determined as follows:

$$r = \text{counts min}^{-1} \text{ for volume filtered} \times \frac{\text{volume of incubation bottle}}{\text{volume filtered}}$$

R = activity added (uc) x dpm uc^{-1} ^{14}C x counter efficiency.

C = total available stable inorganic carbon:

$$C = A\ k$$

where A = total alkalinity (mg $CaCO_3$ l^{-1}), and k is a factor derived from the table of Saunders et al. (1962) based on calculated dissociation constants of H_2CO_3, HCO_3^{-1}, and CO_3^{-2}, pH, and sample temperature (C).

f = a correction factor for discriminatory uptake of the lighter ^{12}C isotope over the slightly heavier ^{14}C radioisotope. Because $^{14}CO_2$ has a molecular weight 4.5% greater than $^{12}CO_2$, the heavier isotope is assimilated at a lesser rate than the lighter. The 6% correction suggested by Goldman (1963) was applied in this investigation.

APPENDIX E

Summary of analysis of variance of the stated variable (i) between the stations specified and (ii) between successive sampling dates at those stations. Stations are located in the Hog Creek Arm (HC), the North Bosque Arm (NB), and the main body (MR) of Waco Reservoir. An asterisk (*) indicates that the calculated F value is statistically significant (P = 0.05) for the appropriate degrees of freedom.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
PHOTOSYNTHETIC EFFICIENCY				
	HC vs. NB			
Between stations	4.96	1	4.96	33.07*
Between sampling dates	11.20	9	1.24	8.27*
Residual	1.34	9	0.15	
Total	17.50	19		
	HC vs. ML			
Between stations	2.28	1	2.28	6.71*
Between sampling dates	14.00	9	1.56	4.59*
Residual	3.11	9	0.34	
Total	19.39	19		
	NB vs. ML			
Between stations	0.52	1	0.52	1.08
Between sampling dates	12.35	9	1.37	2.85
Residual	4.32	9	0.48	
Total	17.19	19		

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
PRIMARY PRODUCTION m^{-2}				
	HC vs. NB			
Between stations	22.11	1	22.11	16.14*
Between sampling dates	53.49	9	5.94	4.34*
Residual	12.33	9	1.37	
Total	87.93	19		
	HC vs. ML			
Between stations	8.34	1	8.34	6.84*
Between sampling dates	33.41	9	3.71	3.04
Residual	11.00	9	1.22	
Total	52.75	19		
	NB vs. ML			
Between stations	3.30	1	3.30	1.22
Between sampling dates	56.91	9	6.32	2.34
Residual	24.30	9	2.70	
Total	84.51	19		

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
PRIMARY PRODUCTION m^{-3}				
	HC vs. NB			
Between stations	5.57	1	5.57	9.60*
Between sampling dates	16.15	9	1.79	3.09
Residual	5.18	9	0.58	
Total	26.90	19		
	HC vs. ML			
Between stations	0.19	1	0.19	0.86
Between sampling dates	3.03	9	0.34	1.54
Residual	2.03	9	0.22	
Total	5.25	19		
	NB vs. ML			
Between stations	7.86	1	7.86	6.83
Between sampling dates	10.36	9	1.15	1.00
Residual	10.39	9	1.15	
Total	28.61	19		

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
	TURBIDITY			
	HC vs. NB			
Between stations	1121.37	1	1121.37	11.02*
Between sampling dates	2277.67	5	455.53	4.478
Residual	508.63	5	101.73	
Total	3907.67	11		
	HC vs. ML			
Between stations	1656.75	1	1656.75	8.25*
Between sampling dates	979.75	5	196.00	0.98
Residual	1003.75	5	200.75	
Total	3640.25	11		
	NB vs. ML			
Between stations	52.09	1	52.09	0.19
Between sampling dates	489.42	5	97.88	0.36
Residual	1345.41	5	269.08	
Total	1886.92	11		

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
PHOSPHATE-PHOSPHORUS				
	HC vs. NB			
Between stations	0	1	0	0
Between sampling dates	46.68	9	5.19	8.37*
Residual	5.57	9	0.62	
Total	52.25	19		
	HC vs. ML			
Between stations	5.41	1	5.41	4.87
Between sampling dates	22.91	9	2.55	2.30
Residual	10.02	9		
Total	38.34	19		
	NB vs. ML			
Between stations	7.51	1	7.51	9.51*
Between sampling dates	27.48	9	3.05	3.86*
Residual	7.12	9	0.79	
Total	42.11	19		

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
FREE CARBON DIOXIDE				
	HC vs. NB			
Between stations	0.02	1	0.02	0.30
Between sampling dates	7.70	9	0.86	12.97*
Residual	0.59	9	0.07	
Total	8.31			
	HC vs. ML			
Between stations	0.01	1	0.01	0.20
Between sampling dates	3.96	9	0.44	8.8*
Residual	0.45	9	0.05	
Total	4.42	1		
	NB vs. ML			
Between stations	0.04	1	0.04	0.32
Between sampling dates	6.1	9	0.68	5.47*
Residual	1.12	9	0.12	
Total	7.26			

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
	SILICA			
	HC vs. NB			
Between stations	0	1	0	0
Between sampling dates	12.33	2	6.16	123.30*
Residual	0.10	2	0.05	
Total	12.43	1		
	HC vs. ML			
Between stations	0.03	1	0.03	0.33
Between sampling dates	9.98	2	4.99	55.44*
Residual	0.18	2	0.09	
Total	10.19			
	NB vs. ML			
Between stations	0.08	1	0.08	1.23
Between sampling dates	10.56	2	5.28	81.23*
Residual	0.13	2	0.06	
Total	10.77			

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
	SULFATE			
	HC vs. NB			
Between stations	8.1	1	8.1	1.10
Between sampling dates	126.6	4	31.65	4.31
Residual	29.4	4	7.35	
Total	164.1			
	HC vs. ML			
Between stations	48.4	1	48.4	4.15
Between sampling dates	69.4	4	17.35	1.49
Residual	46.6	4	11.65	
Total	164.4			
	NB vs. ML			
Between stations	16.9	1	16.9	5.36
Between sampling dates	37.0	4	9.25	2.94
Residual	12.6	4	3.15	
Total	66.5			

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
PHYTOPLANKTON STANDING CROP				
	HC vs. NB			
Between stations	236.50	1	236.5	1.68
Between sampling dates	779.40	3	259.80	1.85
Residual	422.15	3	140.72	
Total	1438.05			
	HC vs. ML			
Between stations	2301.82	1	2301.82	1.24
Between sampling dates	4898.08	3	1632.69	0.88
Residual	5547.86	3	1849.29	
Total	12747.76			
	NB vs. ML			
Between stations	1060.28	1	1060.28	0.47
Between sampling dates	4794.3	3	1598.1	0.708
Residual	6771.7	3	2257.2	
Total	12626.3			

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
DIATOM STANDING CROP				
	HC vs. NB			
Between stations	53.82	1	53.82	2.28
Between sampling dates	214.30	3	71.43	3.03
Residual	70.74	3	23.58	
Total	338.86			
	HC vs. ML			
Between stations	90.45	1	90.45	1.83
Between sampling dates	90.10	3	30.03	0.61
Residual	147.86	3	49.29	
Total	328.41			
	NB vs. ML			
Between stations	4.73	1	4.73	0.06
Between sampling dates	213.79	3	71.26	0.83
Residual	255.19	3	85.06	
Total	473.71			

APPENDIX F

Summary of analysis of variance of carbon fixation (i) at 100% and 75% surface illumination and (ii) between successive sampling dates at these illumination levels at each Waco Reservoir station. An asterisk (*) indicates that the calculated F value is statistically significant (P = 0.05) for the appropriate degrees of freedom.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
	Hog Creek Arm			
Between levels	1.15	1	1.15	13.58*
Between sampling dates	9.21	9	1.02	12.04
Residual	0.76	9	0.08	
Total	11.12	19		
	North Bosque Arm			
Between levels	0.51	1	0.51	1.15
Between sampling dates	22.85	8	2.86	6.41*
Residual	3.56	8	0.44	
Total	26.92	17		
	Main Body of Reservoir			
Between levels	1.43	1	1.43	8.84*
Between sampling dates	4.77	10	0.48	2.95
Residual	1.61	10	0.16	
Total	7.81	21		

APPENDIX C_1

Summary of analysis of variance (i) of effects of experimental nutrient enrichment of primary production per unit volume, and (ii) between successive sampling dates at each Waco Reservoir station. An asterisk (*) indicates that the calculated F value is statistically significant (P = 0.05) for the appropriate degrees of freedom.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
	Hog Creek Arm			
Between treatments	0.17	1	0.17	1.13
Between sampling dates	10.78	4	2.70	18.00*
Residual	0.60	4	0.15	
Total	11.55	9		
	North Bosque Arm			
Between treatments	2.42	1	2.42	0.88
Between sampling dates	93.43	5	18.69	6.77*
Residual	13.78	5	2.76	
Total	109.63	11		
	Main Body of Reservoir			
Between treatments	1.35	1	1.35	19.29*
Between sampling dates	19.37	6	3.23	46.14*
Residual	0.44	6	0.07	
Total	21.16	13		

APPENDIX G_2

Summary of analysis of variance (i) of effects of experimental nutrient enrichment on primary production per unit surface area, and (ii) between sampling dates at each Waco Reservoir station. An asterisk (*) indicates that the calculated F value is statistically significant (P = 0.05) for the appropriate degrees of freedom.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F
	Hog Creek Arm			
Between treatments	0.36		0.36	0.13
Between sampling dates	80.61	4	20.15	7.41*
Residual	10.88	4	2.72	
Total	91.85	9		
	North Bosque Arm			
Between treatments	10.72	1	10.72	0.60
Between sampling dates	272.06	5	54.41	3.04
Residual	89.35	5	17.87	
Total	372.13	11		
	Main Body of Reservoir			
Between treatments	5.52	1	5.52	2.63
Between sampling dates	296.30	6	49.38	23.51*
Residual	12.63	6	2.10	
Total	314.45	13		

APPENDIX H

Partial correlation analysis summary of the relationship between a dependent variable (X_1) and an independent variable (X_2) with the confounding effect of a second independent variable (X_3) held constant. Variables X_1, X_2, and X_3 are stated for each relationship. An asterisk (*) indicates that the calculated t value is statistically significant ($P = 0.05$) for N-3 degrees of freedom. Stations are the Hog Creek arm (HC), the North Bosque arm (NB), and the main body (MR) of Waco Reservoir.

Station	Linear Correlation Coefficient (r_{12})	Partial Correlation Coefficient ($r_{12.3}$)	Percent Linear Association Due to Effects of X_3
a. Photosynthetic efficiency (X_1) vs. Free CO_2 (X_2): X_3=turbidity.			
HC	+0.991*	+0.998*	0
NB	+0.815	+0.901*	0
MR	-0.248	+0.430*	0
b. Primary production m^{-2} (X_1) vs. Free CO_2 (X_2): X_3=turbidity.			
HC	-0.792*	-0.557*	51
NB	-0.593	-0.558*	1
MR	-0.829	-0.928	0
c. Photosynthetic efficiency (X_1) vs. Phosphate-phosphorus (X_2): X_3=turbidity.			
HC	-0.693	-0.448*	58
NB	-0.264	-0.086	90
MR	-0.163	+0.199	0

Station	Linear Correlation Coefficient (r_{12})	Partial Correlation Coefficient ($r_{12.3}$)	Percent Linear Association Due to Effects of X_3
d. Photosynthetic efficienty (X_1) vs. Sulfate (X_2): X_3=turbidity.			
HC	-0.919*	-0.789*	28
NB	-0.862*	-0.843*	4
MR	+0.313	+0.533*	0
e. Photosynthetic efficiency (X_1) vs. Number of identifiable phytoplankters (X_2): X_3=number of diatoms.			
HC	+0.944	-0.433	100
NB	+0.589	+0.238	84
MR	+0.856	+0.415	76
f. Primary production m^{-2} (X_1) vs. Number of identifiable phytoplankters (X_2): X_3=number of diatoms.			
HC	+0.980	-0.600	100
NB	+0.333	+0.273	33
MR	+0.710	+0.535	43
g. Primary production m^{-3} (X_1) vs. Number of identifiable phytoplankters (X_2): X_3=number of diatoms.			
HC	+0.974	-0.289	100
NB	+0.926	_0.240	93
MR	+0.698	+0.578	32

Station	Linear Correlation Coefficient (r_{12})	Partial Correlation Coefficient ($r_{12.3}$)	Percent Linear Association Due to Effects of X_3
h. Photosynthetic efficiency (X_1) vs. Number of diatoms (X_2): X_3=silica.			
HC	+0.962	-0.800*	100
NB	+0.584	-0.966*	100
MR	+0.823	+0.464	68
i. Primary production m^{-2} (X_1) vs. Number of diatoms (X_2): X_3=silica.			
HC	+0.992	+0.999*	0
NB	+0.326	-0.999*	100
MR	+0.616	+0.198	90

APPENDIX I_1

Phytoplankton from Hog Creek Arm nannoplankton samples, August - November, 1968. Data expressed as organisms (10^2) per milliliter water.

Division and Genus	Sampling Dates			
	22 Aug.	5 Sept.	13 Oct.	17 Nov.
Chrysophyta				
Melosira	-	2	4	2
Navicula	2	19	-	1
Stephanodiscus	-	24	29	15
Synedra	8	31	19	3
Chlorophyta				
Closteridium	3	-	4	-
Coelastrum	-	2	1	1
Cosmarium	5	-	-	2
Crucigenia	-	-	2	-
Pediastrum	3	1	-	-
Scenedesmus	-	2	6	2
Selenastrum	-	2	2	-
Staurastrum	-	-	1	-
Tetraedron	-	7	3	1
Tetrastrum	-	-	1	-
Cyanophyta				
Anabaena	-	2	1	-
Euglenophyta	5	5	-	3

APPENDIX I_2

Phytoplankton from North Bosque Arm nannoplankton samples, August - November, 1968. Data expressed as organisms (10^2) per milliliter water.

Division and Genus	Sampling Dates			
	22 Aug.	5 Sept.	13 Oct.	17 Nov.
Chrysophyta				
Melosira	3	2	2	2
Navicula	2	6	2	2
Stephanodiscus	17	12	33	24
Synedra	17	208	22	10
Chlorophyta				
Closteridium	8	-	5	-
Closterium	-	-	2	-
Coelastrum	-	-	3	-
Cosmarium	-	2	2	1
Crucigenia	-	-	2	-
Gleocystis	-	-	7	-
Pediastrum	-	-	-	1
Scenedesmus	-	5	9	1
Selenastrum	-	5	-	1
Tetraedron	-	208	1	4
Cyanophyta				
Anabanena	5	2	6	-
Euglenophyta	7	-	1	2
Pyrrophyta				
Peridinium	-	-	-	1

APPENDIX I_3

Phytoplankton from the reservoir main body nannoplankton samples, August - November, 1968. Data expressed as organisms (10^2) per milliliter water.

Division and Genus	Sampling Dates				
	22 Aug.	5 Sept.	15 Sept.	13 Oct.	17 Nov.
Chrysophyta					
Melosira	3	7	-	2	1
Navicula	3	8	-	-	1
Stephanodiscus	12	46	25	43	22
Synedra	208	40	2	23	10
Chlorophyta					
Closteridium	208	-	1	2	-
Coelastrum	-	2	-	4	2
Cosmarium	-	2	1	2	-
Crucigenia	-	-	-	4	-
Pediastrum	2	2	-	-	-
Scenedesmus	-	14	12	10	2
Selenastrum	-	7	2	1	1
Tetraedron	832	8	12	11	2
Cyanophyta					
Anabanena	2	12	1	4	-
Euglenophyta	7	7	-	1	2

VITA

Bruce Lee Kimmel II was born November 6, 1945 to Bruce and Geraldine Kimmel in Poplar Bluff, Missouri. He attended public schools in Missouri, Texas, Alabama, and Arkansas; and graduated from Jonesboro High School, Jonesboro, Arkansas in May, 1963. He began college education in September, 1963 at The Citadel, Charleston, South Carolina; transferred to Baylor University, Waco, Texas in June, 1965; and was awarded the Bachelor of Science degree in biology in May, 1967.

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