A study of the East Gallatin River, Montana, using an algal bioassay (batch method) and some problems encountered
by Daniel Patrick Griffin

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in Botany
Montana State University

Abstract:
Three bioassays were performed before the old primary sewage treatment plant was closed and three bioassays plus a nutrient addition experiment were performed after the new secondary sewage treatment plant began operation. Usefulness of quantitative nutrient data for prediction of quantitative algal production parameters proved limited. Ammonia-nitrogen (mg/1), orthophosphate (mg/1), and the nitrate-nitrogen to orthophosphate ratio were the three most important independent variables. They were responsible for 43% of the variation of total chlorophyll (mg/1), 64% of the variation of carotenoids (mg/1), and 58% of the variation of maximum number of cells per milliliter. Orthophosphate (mg/1) and ammonia-nitrogen were responsible for 59% of the variation observed in the maximum specific growth rate.

No decisive evidence was obtained on a limiting nutrient, although the choice was between ammonia-nitrogen and orthophosphate.

Some problems limiting the usefulness of this bioassay were discussed.

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Signature Daniel R. Griffith

Date December 2, 1971

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by

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

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Botany

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ABSTRACT

Three bioassays were performed before the old primary sewage treatment plant was closed and three bioassays plus a nutrient addition experiment were performed after the new secondary sewage treatment plant began operation. Usefulness of quantitative nutrient data for prediction of quantitative algal production parameters proved limited. Ammonia-nitrogen (mg/l), orthophosphate (mg/l), and the nitrate-nitrogen to orthophosphate ratio were the three most important independent variables. They were responsible for 43% of the variation of total chlorophyll (mg/l), 64% of the variation of carotenoids (mg/l), and 58% of the variation of maximum number of cells per milliliter. Orthophosphate (mg/l) and ammonia-nitrogen were responsible for 59% of the variation observed in the maximum specific growth rate.

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INTRODUCTION

Ecology, environment and eutrophication are now practically household words, and if all the recent concern and clamor over problems of pollution and the environment seem sudden, the underlying solutions to such problems do not. For example, the problems of surface water eutrophication, its implications and control, have been studied for many years. Instrumental in our knowledge of the process of eutrophication is the algal bioassay procedure for determining potential water fertility. This process in one form or another is not new. Allen and Nelson (1910) did much to develop it with their work on the artificial culture of marine plankton. Atkins (1923), in his study of the relationships between phosphate concentrations and the growth of algal plankton, was also instrumental in developing a algal bioassay technique. Probably the most influential of the earlier investigators was Schreiber (1927). Using a species of Carteria and observing its growth in sample water, he determined the phosphorus and nitrogen concentration of the water and determined which combination of salts was the limiting nutrient factor for its productivity.

In the past 15 to 20 years others have used some form of the algal bioassay procedure for determing the effect of pollution on surface waters (Lackey and Sawyer, 1945; Potash, 1956; Oswald, 1960; Fitzgerald, 1969). Because of this proliferation of procedures, correlation or comparison of data collected has been difficult at best. For this reason a standard algal bioassay procedure (The Provisional Algal Assay

Procedure or PAAP, 1969) has been proposed by a team of international experts under the sponsorship of the Joint Industry/Government Task Force on Eutrophication.

This PAAP is by no means standard procedure; only a set of guidelines around which to build a standard procedure. It includes suggested guidelines not only for the batch or bottle test but also for the continous flow bioassay. This study is concerned with the bottle bioassay.

The East Gallatin River at Bozeman, Montana provides an excellent opportunity for applying the basic concepts of the bottle test. During the course of study the major pollutant (the sewage outfall of the Bozeman City Sewage Treatment Plant) was moved from the old primary treatment plant site 0.7 km (0.4 mi) below the confluence of Bozeman and Rocky Creek to the new secondary treatment plant site approximately 5.4 km (3.4 mi) downstream.

This action provided the unique opportunity of conducting a before and after study on this portion of the river to further assess the effects of pollution on eutrophication, and at the same time assess the problems surrounding the bottle test as a method of determing the potential primary productivity of a water.

DESCRIPTION OF THE STUDY AREA

The East Gallatin River (formed by the confluence of Rocky Creek and Bozeman Creek approximately 0.8 km (0.5 mi) north of Bozeman, Montana) flows in a northwesterly direction 59.5 km (37.0 mi) where it empties into the West Gallatin River. Its drainage area, rich in livestock grazing, wheat farming, and haying, comprises nearly 384.8 km^2 (148 mi^2) at an elevation of about 1,433 m (4,701 ft) above mean sea level.

The average discharge of the East Gallatin River between 1939 and 1961 (measured approximately 0.2 km (0.1 mi) below the confluence of Rocky and Bozeman Creeks) was 2.40 m^3/sec (84.7 cfs). Fluctuations between a fall minimum of approximately 0.51 m^3/sec (18 cfs) and a spring maximum of 5.35 m^3sec (189 cfs) were common. The maximum recorded discharge was 35.14 m^3/sec (1,230 cfs) on June 4, 1953, and the minimum was 0.34 m^3/sec (12 cfs) on December 9, 1944, and March 24-26, 1955, (U.S.G.S., 1964).

During low water the East Gallatin River varies in width from 2.13 m (7 ft) to 9.15 m (30 ft) and in depth from a few inches in the riffles to greater than 2 m (7 ft) in pools. The substrate of the river is composed mainly of cobbles and coarse to fine gravel. The river water is of the calcium-magnesium bicarbonate type.

At a distance of 0.64 km (0.4 mi) downstream from its origin the East Gallatin River is complemented by sewage effluent from the old Bozeman Primary Sewage Treatment Plant (before December 1970). This outfall, an average of 0.14 m^3/sec (5 cfs), comprised from 10 to 20% of the total

river discharge below the effluent during periods of minimum winter flows.

At a point 1.0 km (0.6 mi) below the sewage effluent and 2.0 km (1.2 mi) below the Bozeman and Rocky Creeks confluence, Bridger Creek, a major tributary, enters the East Gallatin River. It has an average discharge of 1.06 m^3/sec (37.6 cfs) and, like Bozeman and Rocky Creeks, drains the surrounding mountainous terrain. Two minor tributaries, Middle Creek and Middle Cottonwood Creek, flow into the East Gallatin River approximately 16.4 km (9.8 mi) downstream from its origin. The flow of the river is also augmented by numerous springs located along its course.

As of December 2, 1970, the sewage effluent from the old Bozeman Primary Sewage Treatment Plant was piped to the site of the new Bozeman Secondary Sewage Treatment Plant. This effluent, which was used to keep the tanks at the new plant from freezing, was discharged into the river at the new site 5.4 km (3.4 mi) downstream from the old plant outfall. On December 15, 1970, priming at the new plant began. The new primary unit received raw sludge for gradually increasing time periods starting with 15 minutes a day while the old primary plant processed the remaining raw sewage as usual. The new secondary unit received the combined effluent from both the new and old primary units until March 15, 1970, when the old primary plant was shutdown completely and the new sewage plant was in complete operation. However, due to mechanical difficulties, the secondary portion of the new plant has not been 100% effective as of this writing.

The six sampling stations established along the East Gallatin River were as follows: (See Figure 1).

Station I - was located 9 m (30 ft) above the old sewage plant outfall before December 1970 and was moved 1.3 km (0.2 mi) downstream to former Station II after December 1970. Both stations were not maintained throughout the study because of limited incubator space.

Station II - before December 1970 was located 0.3 km (0.2 mi) below the old sewage plant outfall at a point where the waste effluent was thoroughly mixed with river water and was moved 5.4 km (3.4 mi) downstream after December 1970. This new point of complete mixing of sewage effluent with river water is 0.3 km (0.2 mi) below the new sewage plant outfall.

Station III - was located 2.2 km (1.4 mi) below the old sewage plant outfall at Manley's Bridge. This station was 1.2 km (0.7 mi) below the mouth of Bridger Creek.

Station IV - was located 5.3 km (3.3 mi) below the old sewage plant outfall approximately 0.16 km (0.1 mi) above the new sewage plant outfall.

Station V - was located 17.4 km (10.8 mi) below the old sewage plant outfall and 12.3 km (7.4 mi) below the new sewage plant outfall.

Station VI - was located 23.5 km (14.6 mi) below the old sewage plant outfall and 18.6 km (11.2 mi) below the new sewage plant outfall.

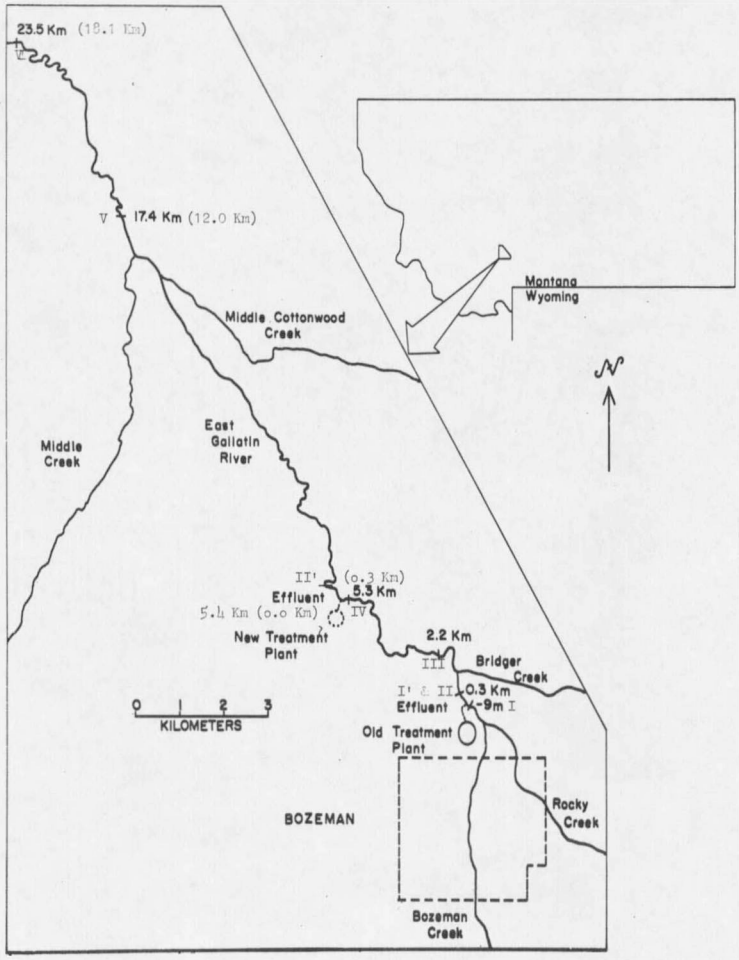

Figure 1. Map of the upper East Gallatin River system showing the location of sampling stations and effluents of the old and new sewage treatment plants. Mileage downstream from new treatment plant outfall is shown in parentheses.

METHODS

Water samples for chemical analyses and bioassay purposes were taken at each of the six stations on the dates shown in Table 1.

All sample water was collected in twice-rinsed standard 2.5 liter (0.7 gal) glass acid jugs with plastic caps. The jugs were completely immersed in the river and filled slowly to maximum capacity, capped and immediately returned to the laboratory for chemical analyses and bioassay procedure.

Water Chemistry

Specific conductance, pH, and total alkalinity were determined for all samples within one hour after returning to the laboratory. The electrical resistance of each sample was measured with a YSI Conductivity Bridge (Model 31) having an Industrial Instruments (Model CEL 4) dipping cell. The cell constant of the dipping cell was approximately 2.05 during the study. The specific conductance of the water at 25°C was computed from the observed resistance corrected for temperature and cell resistance. A Beckman Expanded Scale pH meter (Model 76) was used for pH measurements. Total alkalinity was determined as described by the American Public Health Association (1965).

A portion of each sample was filtered through a 0.45 micron Millipore(R) filter (pretreated with 50 ml of distilled water) and analyzed for poly- and orthophosphates, and ammonia-nitrogen as described by the American Public Health Association (1965). Nitrate- and nitrite-nitrogen were analyzed as described by the Hach Chemical Company (1967). A Klett-Summerson colorimeter was used to measure light extinction in the

Table 1. Sample dates and sewage outfall distances for the established stations.

STATION	DATE	*DISTANCE FROM OLD OUTFALL	NEW OUTFALL
I	10-09-70 10-28-70 11-09-70	-9 m	-5.4 km
I'	1-20-71 2-23-71 3-28-71	0.3 km	-5.1 km
II	10-09-70 10-28-70 11-09-70	0.3 km	-5.1 km
II'	1-20-71 2-23-71 3-28-71	5.1 km	0.3 km
III	10-09-70 10-28-70 11-09-70 1-20-71 2-23-71 3-28-71	2.2 km	-3.2 km
IV	same as above	5.3 km	-0.16 km
V	same as above	17.4 km	12.3 km
VI	same as above	23.5 km	18.6 km

*Minus sign refers to upstream location.

various analyses.

Bioassay

The remaining water from each of the six samples was split into three 400 ml aliquots and filtered through 0.45 micron Millipore(R) filters. This step was preceeded by prefiltering whenever necessary. Each aliquot was then poured into a 500 ml Erlenmyer flask, capped with aluminum foil, and placed in a cooler at 5-10°C until inoculation time. The samples were kept in the cooler between 20 and 45 hours depending on the time they were filtered. Stations were always filtered in numerical order.

Two to three hours before inoculation the samples were taken from the cooler and allowed to warm to room temperature. They were then inoculated with an amount of stock Selenastrum capricornutum necessary to obtain an initial cell concentration of 1,000 cells/ml of sample. All samples were inoculated not more than 54 hours after collection.

The algal cultures were incubated in a Lab-Line Instruments, Inc. incubator, catalog no. 844, (see Figure 2) at 150-190 foot-candles and at 24 $\pm$ 1°C. Continuous bubbling served to agitate the algae and aerate the sample.

As soon as algal growth was visible, and everyday thereafter at approximately the same time, the sides of the sample flasks were rubbed with a rubber policeman and the samples were stirred with a magnetic stirrer. This was also done immediately prior to sample counting which