Technical Progress Report

Microbiology of Thermally Polluted Environments

Contract Period: August 1, 1972 - July 31, 1973

Date of Report: April 1, 1973

Principal Investigator:

Thomas D. Brock

Department of Bacteriology

University of Wisconsin

Madison, Wisconsin 53706

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

Thomas D. Brock

Thomas D. Brock
Principal Investigator

PREPARED FOR THE U.S. ATOMIC ENERGY COMMISSION UNDER

CONTRACT NO. AT(11-1)-2161

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

leg

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

I. Introduction

This report provides details of research carried out on this contract during part of the fourth and fifth years of its existence. Several papers concerning work supported in part or fully by this contract have been published, and reprints are attached. Other work in press or in manuscript form is included in the narrative of this report.

This report describes some of the initial studies undertaken after the Principal Investigator had moved to the University of Wisconsin-Madison. The Madison location has permitted studies on thermal effects on lakes, using techniques and concepts developed from our previous Yellowstone work. In addition, the Yellowstone work has been considerably extended, especially the work on thermal effects on the Firehole River.

The Principal Investigator spent about 10% of his time on this project. Other personnel involved in these studies were Dr. Charles Boylen and Dr. Ben Bohlool, Research Associates Kathryn Boylen and James Hoffman, Research Specialist Bonnie Bauld, and Student Assistants Beverly Thompson, Katherine Sprague, Germaine Juneau, and Sandra Peterson. Several other individuals in this laboratory made contributions to this project although they were supported by funds from other sources. These include Dr. Robert Belly (N.I.H. post-doctoral fellow), Dr. Jerry Messer (Senior Research Associate), David Smith, John Bauld, and David Ward (graduate students).

Publications of AEC Contract AT(11-1)-1804

- C00-1804-1 Technical Progress Report 1968.
- C00-1804-2 Brock, T.D. and M.L. Brock. 1969. Recovery of a hot spring community from a catastrophe. *Journal of Phycology* 5: 75-77.
- C00-1804-3 Brock, M.L., R.G. Wiegert and T.D. Brock. 1969. Feeding by Paracoenia and Ephydra (Diptera: Ephydridae) on the microorganisms of hot springs. *Ecology* 50: 192-200.
- C00-1804-4 Brock, T.D. and M.L. Brock. 1969. The fate in nature of photosynthetically assimilated ^{14}C in a blue-green alga. *Limnology and Oceanography* 14: 604-607.
- C00-1804-5 Bott, T.L. and T.D. Brock. 1969. Bacterial growth rates above 90°C in Yellowstone hot springs. *Science* 164: 1411-1412.
- C00-1804-6 Brock, T.D. and H. Freeze. 1969. Thermus aquaticus gen. n. and sp. n., a non-sporulating extreme thermophile. *Journal of Bacteriology* 98: 289-297.
- C00-1804-7 Brock, T.D. and M.L. Brock. 1969. Effect of light intensity on photosynthesis by thermal algae adapted to natural and reduced sunlight. *Limnology and Oceanography* 14: 334-341.
- C00-1804-8 Brock, T.D. One hundred years of algal research in Yellowstone National Park. First International Symposium on Blue-green Algae. Madras, India. (In press).
- C00-1804-9 Not published.
- C00-1804-10 Bott, T.L. and T.D. Brock. 1970. Growth and metabolism of periphytic bacteria: methodology. *Limnology and Oceanography* 15: 333-342.
- C00-1804-11 Bott, T.L. and T.D. Brock. 1970. Growth rate of Sphaerotilus in a thermally polluted environment. *Applied Microbiology* 19: 100-102.

- C00-1804-12 Technical Progress Report 1969.
- C00-1804-13 Doemel, W.N. and T.D. Brock. 1970. The upper temperature limit of Cyanidium caldarium. Archiv fur Mikrobiologie 72: 326-332.
- C00-1804-14 Brock, T.D. and M.R. Edwards. 1970. Fine structure of Thermus aquaticus, an extreme thermophile. Journal of Bacteriology 104: 509-517.
- C00-1804-15 Brock, T.D. 1970. High temperature systems. Annual Review of Ecology and Systematics 1: 191-220.
- C00-1804-16 Brock, T.D. and G.K. Darland. 1970. Limits of microbial existence: temperature and pH. Science 169: 1316-1318.
- C00-1804-17 Brock, T.D. 1971. Ecological and evolutionary aspects of morphogenesis, p. 77-89. In: Recent Advances in Microbiology. 10th International Congress for Microbiology, Mexico City, Aug. 1970.
- C00-1804-18 Brock, T.D. and M.L. Brock. 1970. The algae of Waimangu Cauldron (New Zealand): distribution in relation to pH. Journal of Phycology 6: 371-375.
- C00-1804-19 Darland, G., T.D. Brock, W. Samsonoff and S.F. Conti. 1970. A thermophilic, acidophilic mycoplasma isolated from a coal refuse pile. Science 170: 1416-1418.
- C00-1804-20 Darland, G. and T.D. Brock. 1971. Bacillus acidocaldarius sp. nov., an acidophilic thermophilic spore-forming bacterium. Journal of General Microbiology 67: 9-15.
- C00-1804-21 Brock, T.D. 1971. Microbial adaptation to extremes of temperature and pH, p. 32-37. In: I.A. Bernstein, ed., Biochemical Responses to Environmental Stress. Plenum Press, N.Y.
- C00-1804-22 Brock, T.D. 1971. Microbial growth rates in nature. Bacteriological Reviews 35: 39-58.
- C00-1804-23 Brock, T.D. and M.L. Brock. Microbiological studies of thermal habitats of the central volcanic region, North Island, New Zealand. New Zealand Journal of Marine and Freshwater Research. (In press).

- C00-1804-24 Brock, T.D. and I. Yoder. 1971. Thermal pollution of a small river by a large university: bacteriology studies. Proceedings of the Indiana Academy of Sciences 80: 183-188.
- C00-1804-25 Mosser, J. and T.D. Brock. 1971. Effect of wide temperature fluctuation on the blue-green algae of Bead Geyser, Yellowstone National Park. Limnology and Oceanography 16: 640-645.
- C00-1804-26 Zeikus, J.G. and T.D. Brock. Effects of thermal additions from the Yellowstone geyser basins on the bacteriology of the Firehole River. Ecology (In press).
- C00-1804-27 Brock, T.D. 1971. Bimodal distribution of pH values of thermal springs of the world. Bulletin of the Geological Society of America 82: 1393-1394.
- C00-1804-28 Brock, T.D., M.L. Brock, T.L. Bott and M.R. Edwards. 1971. Microbial life at 90 C: the sulfur bacteria of Boulder Spring. Journal of Bacteriology 107: 303-314.
- C00-1804-29 Brock, T.D. and M.L. Brock. 1971. Thermophilic Bacteriology--Physiology of non-sulphur bacteria from hot springs. Nature 233: 494-495.
- C00-1804-30 Technical Progress Report 1970.
- C00-1804-31 Doemel, W.N. and T.D. Brock. 1971. The physiological ecology of Cyanidium caldarium. Journal of General Microbiology 67: 17-32.

Types
removed

Publications supported by AEC contract AT(11-1)-2161

- COO-2161-1 Boylen, C.W. and T.D. Brock. Effects of thermal additions from the Yellowstone geyser basins on the benthic algae of the Firehole River. Ecology, in press.
- COO-2161-2 Brock, T.D. and K.L. Boylen. 1973. Presence of thermophilic bacteria in laundry and hot-water heaters. Appl. Microbiol. 25: 72-85.
- COO-2161-3 Brock, T.D., F. Passman and I. Yoder. Absence of obligately psychrophilic bacteria in constantly cold springs associated with caves in southern Indiana. Amer. Midland Naturalist, in press.
- COO-2161-4 Fliermans, C.B. and T.D. Brock. 1972. Ecology of sulfur-oxidizing bacteria in hot acid soils. J. Bacteriol. 111: 343-350.
- COO-2161-5 Technical Progress Report. 1972.
- COO-2161-6 Boylen, C.W. and T.D. Brock. Bacterial decomposition processes in Lake Wingra sediments during the winter. Limnol. Oceanogr., in press.
- COO-2161-7 Shivvers, D.W. and T.D. Brock. 1973. Oxidation of elemental sulfur by Sulfolobus acidocaldarius. J. Bacteriol., in press.
- COO-2161-8 Belly, R.T., B.B. Bohlool and T.D. Brock. The genus Thermoplasma. Annals N.Y. Acad. Sci., in press. (Invitational Paper).
- COO-2161-9 Technical Progress Report. 1973.

Boylen, C.W. and T.D. Brock. Effects of thermal additions from the Yellowstone geyser basins on the benthic algae of the Firehole River. Ecology, in press.

Reviewed

This major paper has been accepted for publication by Ecology. The manuscript attached differs considerably from that submitted last year, under the same COO number, as it now includes work from two field seasons. Because of the length of this paper, the key conclusions are summarized here.

1. Heating of the river leads to increased populations of benthic algae. The effect is a direct one of temperature, and not an indirect effect of nutrients or other factors. Both the standing crop and the growth rate of the algae are greater in the heated than in the unheated portions of the river.

2. The algae are optimally adapted to the temperatures at which they are living. The controlling temperature is that reached in mid-summer, when the river has warmed to its maximum extent because of both solar heating and heating by thermal additions from the geyser basins.

3. The algae retain their summer temperature optima even during the cold period of the spring run-off. Seasonal changes in the temperature optima of the algal populations do not occur. The algae are thus "pre-adapted" to the mid-summer temperatures throughout the whole year. Because of this, the low algal standing crop during the cold period of the spring run-off probably occurs because the algae are unable to grow at peak efficiency. A corollary of this is that the increased standing crop and growth rate during the summer is a direct effect of heating.

4. Preliminary work (described elsewhere in this report) suggests that a similar situation occurs in natural waters receiving thermal additions from

power plants. Taken together, these results provide the first direct evidence that thermal additions cause increases in algal growth and standing crop.

They make it possible to predict the consequences of thermal pollution. They suggest that it is probably only during the critical mid-summer period that thermal additions must be completely avoided, if the algal populations are to remain in their natural state.

Effects of Thermal Additions from the Yellowstone
Geyser Basins on the Benthic Algae of the
Firehole River

Charles W. Boylen¹ and Thomas D. Brock

Department of Bacteriology, University of Wisconsin, Madison 53706

• ¹ Present address: Department of Biology, Rensselaer Polytechnic
Institute, Troy, New York 12181

Abstract

During the spring and summer of 1971 and 1972 quantitative measurements on temperature, water chemistry, and standing crop of benthic algal mats were made at a series of stations along the Firehole River as it flows through the main geyser basins of Yellowstone National Park. The river is viewed as an ideal study area for assessing the long-term effects of thermal additions, since it has been subject to heating effects for hundreds of years. Hot springs and geysers in the geyser basins add to the river heated and nutrient-enriched water of about one-third the volume of river water. As the river flowed through the thermal areas, the temperature gradually increased to a maximum daily midsummer temperature 12°C higher than the unheated upper portion of the river (15° to 27°C) and the river began cooling when the thermal additions to the river ceased. The river was coldest in early June, when maximum input from melting snow occurred, and remained relatively warm in the heated areas throughout the winter. Alkalinity, pH, conductivity, phosphate, and chloride also increased markedly; nitrate and ammonium concentrations appeared not to be influenced by the additions of hot spring water. The quantity of chlorophyll per unit area extracted from epilithic algal mats also increased with the amount of thermal water input to a value 20-fold higher than in the unheated control areas. The apparent growth rates of benthic algae calculated from periodic

quantitative sampling of cleaned rocks were over 5-fold greater in the warmer than in the cooler portion of the river. The effect of temperature on light-dependent incorporation of $^{14}\text{CO}_2$ by epilithic, epipellic, and epiphytic algal communities at different stations was measured. The temperature optimum for CO_2 fixation paralleled the mid-summer temperature of the habitat although it averaged 3 degrees higher at each station. The algae retained their mid-summer temperature optima even during the early June period of cold water, suggesting that it is the mid-summer maximum temperature which controls algal temperature optima. Measurement of photosynthesis with natural populations was made in the presence of increased concentrations of the algal nutrients to determine whether the increased algal growth rate in the warmer parts of the river might be due to nutrient enrichment rather than to temperature. These results suggest that increases in temperature from the additions of thermal water increase the algal standing crop through increase in growth rate of the algae, and that the algae in the heated portion of the river are optimally adapted to the temperatures of their habitats.

INTRODUCTION

The Firehole River in Yellowstone National Park, Wyoming, a world famous trout stream, makes a good model for studying the ecological consequences of thermal additions. First, the thermal additions involve a natural rather than a man-influenced aquatic ecosystem and one which has presumably remained relatively constant over many years. Secondly, the rise in temperature in the warmest portion of the river is comparable to temperature increases caused by electrical power plants in many parts of the world (Parker and Krenkel, 1969; Eisenbud and Gleason, 1969). One presumed consequence of thermal pollution of natural waters is an increase in algal growth and a change in the algal species present (Patrick, 1969). However, considerably more work has been done on the effect of temperature on the kinds of algal species than on the standing crop and productivity of whole algal communities. The purpose of the present work was to study standing crop and growth rate of benthic algae in the heated and unheated sections of the Firehole River, and by use of radioisotope techniques to measure the temperature optima of the natural algal populations to determine whether adaptation to the various environmental temperatures had occurred. This work supplements and extends that of Zeikus and Brock (1972), which dealt with the bacterial populations of the Firehole River. The results reported here are based on work over two years, 1971 and 1972. Except for minor differences, the findings of both years were similar, and we

present here for each portion of the study the data from that year which is most complete.

The extent of the thermal additions to the Firehole River was measured by Allen and Day (1935). The total discharge from the geyser basins into the Firehole River was $1.74 \text{ m}^3/\text{sec}$ ($61.5 \text{ ft}^3/\text{sec}$); whereas the volume of flow of the river itself was $3.82 \text{ m}^3/\text{sec}$ ($135 \text{ ft}^3/\text{sec}$). Although there were changes in certain thermal activities in the park following the earthquake of 1959 (Fischer, 1960), the total thermal input into the river has apparently remained essentially the same since Allen and Day's measurements. The thermal water received by the river is sufficient to raise the temperature (below mixing zones) in summer approximately $12^\circ\text{-}14^\circ\text{C}$. The physical processes involved in the mixing of thermal water with Firehole River water have been studied by Argyle (1966).

Temperature measurements and chemical analyses of heated and unheated sections of the river by Armitage (1958), Wright and Horrall (1967), and Zeikus and Brock (1972) have been roughly comparable, and compare favorably to our own data. However, we have carried out more detailed temperature studies with some attention to seasonal and diurnal variations, and have assayed several chemical entities of biological importance not previously assayed. We have measured growth rate and standing crop of benthic algae at a series of stations along the river, and by use of radioisotope techniques have measured the temperature optimum for photosynthesis of the algae at each station.

Our results show that growth rate and standing crop are higher in the heated portions of the river, and that the algae are optimally adapted to the temperature of their habitat.

MATERIALS AND METHODS

Selection of stations

The location of the eight sampling stations on the Firehole River were given by Zeikus and Brock (1972). The stations covered a distance of 35 km and each will be subsequently referred to in the text by Roman numeral. For the present work, a new station X was located 1.4 km downstream from the original station X in a region where the bottom substrate was more favorable for algal growth. Due to changes in the road system around the Old Faithful area it was inconvenient to use the station II of Zeikus and Brock, but this station had little heating and its elimination was hence not critical. Continued road alterations in this area eliminated the use of station III during the summer of 1972. Station IX is in the Gibbon River above the confluence with the Firehole River and was sampled randomly throughout the summer, 1971, for temperature only. The stations were located such that water and algal samples were taken from (a) an area where there is negligible thermal input (station I), (b) areas of major thermal input (stations III through VIII), and (c) a recovery area in the Madison River 10.5 km downstream from the junction of the Gibbon and Firehole Rivers where there is no further thermal input (station X). Stations were always located downstream from areas of

rapid mixing (e.g., below riffle areas); at any station the water temperature was constant across the river. It should be noted that there is some altitude difference between some of the stations: station I (near 2300 M); stations II-VII (2200-2050 M); stations VIII-X (about 2000 M).

Light intensity was virtually the same at all stations and mid-afternoon intensities averaged 6000 footcandles on sunny days. Bank effects and tree shading of the stations were minimal. Minimum summer river depth was approximately 0.25 m for all stations. Stations were placed so that directional changes in the river channel caused no light effects. Sampling was always done at least 3 m from one shore line.

Routine assays

Water samples for pH, alkalinity, conductivity, phosphate, nitrate, chloride, and ammonium analyses were collected in 1 liter Pyrex bottles with tight-fitting Teflon-lined screwcaps. Analyses were made in a field laboratory in West Yellowstone, Montana, usually on the same day samples were taken. Temperature was measured in situ with a thermistor (Model 42SC, Yellow Springs Instrument Co.). Hydrogen ion concentration was routinely measured in the laboratory with a Corning pH meter. When pH determinations were made in the field, an Orion battery-operated pH meter was used. The potentiometric titration procedure was used to measure alkalinity as described by the American Public Health Association (1965). Samples of 100 ml were titrated with 0.02 N HCl to pH 4.4 using a pH meter. Conductivity was measured using a conductivity bridge fitted

with a 3400 series conductivity cell (Yellow Springs Instrument Co.). Orthophosphate content was determined by the stannous chloride method as described by the American Public Health Association (1965). Nitrate, chloride, and ammonium ions were assayed by Dr. Kimio Noguchi and associates at a field laboratory in West Yellowstone, Montana. Nitrate was reduced to nitrite with zinc dust and the nitrate assayed by diazotization, chloride was determined by titration with silver nitrate using potassium chromate as an indicator, and ammonium was determined by direct nesslerization (American Public Health Association, 1965).

Algal standing crop

Algal standing crop measurements were made at each station during August, 1971 and through May to August, 1972. Chlorophyll a determinations were used as an index of algal biomass. Native stones of approximately 200 cm² were taken from the river, placed in shallow pans, and given several moments for excess water to drain from them. The stones were then placed in heavy plastic bags (Brown-in-bags, Reynolds Metal Co., Richmond, Virginia) top surface down, and acetone (100%, technical grade) added to completely submerge the algal mat. Stones were kept in the dark during transport to the laboratory. As long as the bags were handled gently and were cushioned during transport they held the acetone well and leakage was kept to a minimum. When leakage did occur the bag and its contents were transferred to a second bag. Complete extraction of the chlorophyll was achieved during overnight refrigeration at 4°C. After the volume of acetone extract was determined, absorbancy was measured at 663 nm on a Beckman DB-G spectrophotometer. Chlorophyll a

was calculated using the relationship given by Golterman (1969). Spectral studies of random samples showed no chlorophyll b peak at 650 nm and only a small chlorophyll c peak at 620 nm constituting a peak height of 10% or less of that for chlorophyll a. No peak at 405 nm was seen in unacidified extracts; therefore, it was concluded that negligible pheophytin was present either in the natural material or in the acetone extract. After extraction the stones were allowed to dry and the area of algal mat measured. This could be easily done since the position of the algal mat was visualized by a white residue remaining on the stone. Algal biomass was expressed in terms of $\mu\text{g chlorophyll}/\text{cm}^2$.

Algal growth rate

The apparent growth rates for natural algal communities was determined during the summer, 1971, at all stations and during the summer, 1972, at stations I, VI, and X. Clean smooth stones collected from a glacial outwash plain in the vicinity of the Madison River (west of Earthquake Lake, Montana) were placed in the river in mid-June to provide a reasonably natural substrate for algal growth. These placed stones were marked on the underside with a felt tip pen to insure proper identification. Triplicate stones were removed at approximately weekly intervals and algal biomass determinations made as described above.

Isotope incorporation studies

The optimum temperature for $^{14}\text{CO}_2$ incorporation by suspensions of benthic algae was determined directly in the field. Experimental procedures were patterned after those described by Brock (1967). Epilithic algae were removed from native stones taken from the river bottom and placed in a shallow tray half-filled with river water. The whole top surface of a stone was scrubbed with a plastic brush and the algal suspension placed in a plastic tube and briefly homogenized with a Teflon plunger to break up clumps. Epipellic algae were scooped from the surface of river bottom mud and sand deposits with a 250 ml wide-mouth plastic bottle. Heavy material fell to the bottom of the bottle immediately and the algae remaining in suspension were decanted off. Epiphytic algae were removed from the river macrophytes by gently agitating the plant body in a wide-mouth plastic bottle filled with river water. Both epipellic and epiphytic algal populations were always more loosely bound than epilithic algae and only the slightest homogenization was necessary to break up the flocs. The resultant suspensions were decanted into 250 ml plastic bottles from which 4 ml was dispensed into each screw-capped vial (5.0 ml total volume). The vessel was placed on its side in water several centimeters deep. Incubation temperatures above ambient water temperature were selected from nearby thermal effluent channels; water temperatures below ambient were obtained by using

shallow pans containing ice. Temperatures were monitored at each incubation site and ice added as necessary to maintain the temperatures below ambient. Natural illumination was used in all experiments. After 10 min for thermal equilibration, 0.1 ml of a stock solution of $\text{Na}_2\text{H}^{14}\text{CO}_3$ containing 10 $\mu\text{Ci/ml}$ was added to each vessel and the vessel quickly returned to the incubation site. The activity of isotope used, 1 $\mu\text{Ci/vial}$, was selected to give a reasonable counting rate after processing. Algal suspensions containing 1 to 10 μg chlorophyll/vial were routinely used and rate of uptake was linear for four hours, suggesting that no changes in the population were occurring during incubation. Isotope incorporation was terminated after one hour incubation by the addition of formalin to the vial to give a final concentration of 4% formaldehyde. Aliquots of the suspension were filtered through 0.45 μm membrane filters and the radioactivity determined with a Nuclear-Chicago gas flow counter. Self absorption was not a problem since it was determined that algal suspensions containing up to 25 μg chlorophyll/filter gave no quenching of radioactivity. Radioactivity was expressed as CPM/ μg chlorophyll.

Algal taxonomy

Algal samples for microscopy were taken at each station during the summers of 1971 and 1972. Stones were removed from the river, placed in shallow pans, and the algal material removed using a plastic brush. Suspensions were transferred to plastic bottles and formalin at 4% final concentration added to each. In the laboratory each suspension was placed

in a plastic tube and briefly homogenized with a Teflon plunger to break up clumps. The algae constituting the majority of the biomass were identified to genus from the keys of Prescott (1951) and Smith (1950) using a Zeiss phase microscope.

RESULTS

The temperature and water chemistry data shown in this report were taken during the summer, 1971, when sampling was done routinely at weekly intervals. Additional data on temperature and water chemistry were collected periodically during the summer, 1972, and are similar to the 1971 determinations.

Temperature

The temperatures taken at the various stations throughout 1971 are summarized in Table 1. Temperature ranged from a low of 4.5°C at station I in late spring to a high of 27.0°C at station VI in mid-summer. Those temperatures observed at station I (4.5°C to 14.5°C) are characteristic of unheated mountain streams (McConnell and Sigler, 1959). At station VI, just below the last hot spring water input, temperature was at its highest point, about 12°C higher than at station I. Warming of the river is very prominent throughout the year and was even evidenced in the winter as shown by the temperatures taken January 27, 1971. When there are no further thermal additions to the river, return of the temperature to the upper stream value is not immediate. Station X located on the Madison River at least 14.2 km

downstream from any additional thermal input averages 8 to 12 degrees higher than the temperatures recorded at station I.

The least influence of thermal water is during the late spring when a great volume of cold runoff water from melting snow enters the river. Water temperature is graphed with respect to time during the summer of 1971 for stations I, VI, and X in Fig. 1. The snow-melt water additions diminish to a negligible influence by early or mid-July, and the geyser and hot spring effluents then exert their maximum warming influence upon the water.

The influence of air temperature upon the water temperature during the mid-summer when the water level is at its lowest and air temperatures highest must also be considered. Temperatures were taken at each station over a 24 hr period during August 16 to 17, 1971. All stations exhibited a considerable diurnal change in water temperature. Data for only stations I, VI, and X are shown in Fig. 2. Each station fluctuated 5 to 7 degrees during the 24 hr period, with the coolest temperature reached between 6 and 9 a.m. and the warmest reached between 3 and 6 p.m. Although measurements were not made at other times during the summer, casual observations indicated that diurnal temperature fluctuations were occurring. Most of the routine observations were made in the afternoon when the river would be the warmest. The average temperature for each station during the warm summer period is summarized in Table 2. The highest temperature reached in the river was 27°C at station VI.

Water chemistry

The thermal effluents not only influence the temperature of the river but also its chemical composition as shown in Table 2, which provides data for the mid- and late-summer periods. Alkalinity, phosphate, chloride, and pH markedly increase as the thermal effluents enter the river. However, nitrate and ammonium concentrations appear not to be influenced by the thermal additions. Since the hot spring waters of the main geyser basins are alkaline, high in phosphate and chloride, and low in nitrate and ammonium, these results are understandable.

Spring and early-summer data for the heated stations must be considered separately. Alkalinity, conductivity, and phosphate concentrations are plotted versus date of sampling in Fig. 3. The analyses at all the stations exhibited similar curves so for clarity only the data from stations I, VI and X are plotted. As seen, the mineral concentration of the unheated stretch of the river remains about the same all summer, whereas in the heated sections of the river the concentrations rise in mid-summer, reflecting a decrease in runoff from snow melt. When the river depth reaches a minimum in August, the effects of the thermal additions become maximum and show little further variation.

As the river depth decreased, the pH increased, reflecting a decrease in mildly acidic snow runoff and an increased influence of alkaline thermal water, and an increase of approximately 0.5 to

1.0 pH unit was observed at the stations between June and August. All the stations also exhibited a considerable diurnal change in water pH; for instance, there was a fluctuation of 0.2 units at station I, 0.4 units at station VI, and 0.7 units at station X. The diurnal fluctuations in pH reflect changes in the bicarbonate equilibria due to photosynthetic activity of algal and macrophytic communities (Wright and Mills, 1967).

Algal standing crop and growth rate

The epilithic algal biomass produced from May through August, 1972, was estimated by assaying at periodic intervals the amount of chlorophyll per unit area of algal mats developing on native stones. Determinations on May 23 showed biomass values to be very close to measurements made at the end of August the previous year. River depth was not yet noticeably affected by snow-melt runoff and was approximately the same as the depth in mid-summer. During a three week period extending to June 22 the river flowed at approximately $2/3$ higher than its normal depth. Scouring of the benthic surface took place during this time as reflected in the decrease in standing crop from May 31 through June 22. Thereafter the river level steadily decreased and standing crop measurements began to increase.

It is assumed that the algal biomass removed from native stones on the last sampling date, August 2, 1972, represents an approximation to the maximum standing crop for the summer growing season. As seen in Fig. 4, the amount of algal biomass increases downstream through

the heated section of the river, reaching a maximum of 15 μg chlorophyll/ cm^2 at station VI and then decreasing in the portion of the river where recovery occurs. Thus in the heated section of the river algal standing crop is 15-fold higher than in the cold upstream section.

Apparent algal growth rate (generation time) was more accurately determined from biomass determinations on placed stones than on native stones on which large population densities already existed from the beginning of the summer. During 1971 clean stones were placed at all stations and in 1972 at stations I, VI, and X. Biomass determinations were made from triplicate stones at approximately 10 day intervals. The growth rates for stations I, VI, and X are shown in Fig. 5, and calculated doubling times for all stations are shown in Fig. 4. As can be seen, growth rates were progressively higher in the warmer areas, the rate at station VI being 5-fold higher than that at station I.

Temperature optima of resident algal populations

Studies were carried out to determine whether the algae at different stations were adapted to the temperatures of their habitats. The technique used was similar to that previously described (Brock, 1967) in which the effect of temperature on light-dependent $^{14}\text{CO}_2$ fixation was measured. Most of the studies were done on epilithic populations, but some studies were done on algae obtained from mud surfaces and from the surfaces of macrophytes.

Well-defined temperature optima were obtained for algae from all stations. Typical temperature optima are illustrated in Fig. 6 in which the data obtained with epilithic algal suspensions from stations

I and VI are presented. The optimum temperature for $^{14}\text{CO}_2$ incorporation for all the stations are compared with the maximum daily temperatures of the stations in Fig. 7. As seen, the temperature optima parallel the temperatures of the habitat, although the optima average about 3 degrees higher than the habitat temperatures. The optimum temperature data for $^{14}\text{CO}_2$ fixation by the epipellic and epiphytic algae from stations I and VI were similar to those of the epilithic algae.

To determine whether or not adaptation to lower temperatures of winter and spring occurred, the optimum temperature for $^{14}\text{CO}_2$ incorporation was determined for each epilithic algal community during May and June, 1972. Temperature optima obtained for the algae at each station during late spring showed approximately identical optima to those obtained the previous August, even though habitat temperatures were 10°C lower. These results suggest that seasonal variation in habitat temperatures is not of sufficient duration to allow algal adaptation to the lower temperatures. In fact, it appears that these communities remain adapted to a maximum habitat temperature which coincides with a time of maximum light intensity and greatest productivity.

Nutrient affects on algal photosynthesis

Because of the marked increase in certain algal nutrients in the heated section of the river, it was of interest to determine whether nutrients might be limiting algal growth in the colder section of the river. To this end, some preliminary experiments were done to determine

the effects of added algal nutrients on photosynthesis of populations at station I and station VI as measured with the ^{14}C technique. This approach has been used extensively by Goldman (1965) and Wetzel (1965) to identify nutrients limiting algal growth in natural waters. Experiments were done in which river water was enriched 5- and 10-fold with phosphate, nitrate, ammonium, and bicarbonate. No stimulation of photosynthesis by phosphate, nitrate, or ammonium occurred either at station I or station VI. Addition of bicarbonate did stimulate uptake of $\text{NaH}^{14}\text{CO}_3$ (when the decrease in specific radioactivity of the radioisotope was considered), but the stimulation was even greater at station VI than at station I. These data suggest that although bicarbonate may be limiting algal photosynthesis, this limitation cannot explain the higher algal standing crop and growth rate in station VI than in station I, and that the increased algal growth rate in the warmer sections of the river is at least in part due to increased water temperature. However, these preliminary experiments will have to be extended before any final conclusion can be drawn.

Algal floristic studies

Exhaustive identification of algal species inhabiting the Fire-hole and Madison Rivers have been made by Roeder (1966) and Jones (1967). Since our work dealt with the growth rate and temperature optima of algal communities established at each station, detailed studies on algal species were not warranted, although we identified all significant algae to genus. Indeed, in many cases identification to species would be impossible, since it would require the observation of reproductive structures which were not present. The algal communities which developed at

each station were very similar for both summers and are summarized together.

At station I, which had very little algal biomass, diatoms of the genera Navicula, Synedra, and Nitzschia constituted the majority of the flora of samples taken at any time during the summer. Filaments of the green alga Spirogyra were found in late summer but not in early summer. At stations VIII and X diatoms also constituted most of the biomass, represented primarily by the genera Gomphonema, Cymbella, Nitzschia, Amphora, and Rhopalodia.

At station IV, a mat composed primarily of the blue-green alga Nostoc was seen over the course of both summers. The green alga Cladophora constituted a small portion of the total biomass as well as the diatoms Nitzschia, Gomphonema, Navicula, and Amphora. At stations V and VI, the blue-green algae Oscillatoria, Lyngbya, Phormidium, and Tolypothrix dominated samples taken from old stones at the beginning of summer, and low diatom populations were also present. As the summer progressed, blue-green algae and diatoms constituted a smaller fraction of the total biomass of the developing mats. During late July and August the heavy epilithic mat that developed at stations V and VI consisted primarily of the green filamentous algae Spirogyra, Oedogonium, Cladophora, and Stigeoclonium. Decrease in species diversity as standing crop increases has been noted in diatom communities of the Firehole River by Roeder (1966).

Transitions of algal populations were observed in the studies done on placed stones. After 7 days in the river, diatoms were always the initial colonizers at all stations, the genera being Nitzschia, Synedra,

Rhopalodia, Cocconeis, and Gomphonema. After 40 days in the river diatoms continued to constitute the majority of the biomass at stations I, VIII, and X; however, at the warmer stations III, V, and VI the green algae Spirogyra, Oedogonium, Cladophora and Stigeoclonium dominated.

Epiphytic and epipelic algal communities at station I were very similar to those growing on rocks, and were composed entirely of diatoms. At station VI the epiphytic algae consisted primarily of the green algae Spirogyra and Closterium although the diatoms Amphora, Synedra, and Rhopalodia contributed significantly to the total biomass. Epipelic algal communities at station VI consisted mostly of diatoms of the genera mentioned above. The blue-green algae Oscillatoria and Lyngbya were observed in small quantity but no green algae were found.

DISCUSSION

It is clear from the present work that the addition of thermal water to the Firehole River has marked effects on the benthic algae. The standing crop is 15-fold higher in the heated than in the unheated portion of the river, and the growth rate is over 5-fold greater. In the recovery zone of the river, represented by stations VII and X, both standing crop and growth rate decrease again, but never to the low value found at station I. However, the temperatures of these stations are higher at all times than those of station I, so that a complete recovery to the upstream conditions does not occur within the existing length of the river.

The increased standing crop in the heated portions of the river does conform to expectations derived from a review of the thermal pollution literature (Eisenbud and Gleason, 1969; Flemer, et al., 1970; Krenkel and Parker, 1969; Parker and Krenkel, 1969). The standing crop of algae is a function of three major variables: algal growth rate, washout rate, and grazing by herbivores. Growth rate itself should be a function mainly of temperature, light, and nutrient concentrations. Since light should be virtually the same throughout the length of the river, it is unlikely that this is a variable responsible for varying growth rates at the different stations, although it may be responsible for seasonal differences in growth rate at all the stations.

Washout is probably only a major factor in determining algal standing crop during the spring and early summer when runoff from melting snow greatly increases the scouring action of the river. From mid-summer through winter the water level in the river is low and fairly constant, and scouring is at a minimum. Our studies corresponded mainly with the season when scouring had been completed and the water level was low. The rates of increase in algal biomass which we measured on scoured rocks are probably equivalent to the maximum growth rates which the algae can achieve, since during colonization and growth on barren rocks minimum competition between adjacent algal filaments for nutrients and light should occur.

The third factor affecting standing crop is grazing. In 1971, stones placed in the water became colonized with larvae, but in 1972 such colonization did not occur, although the growth rates and standing crops were

similar both summers. Thus it seems unlikely that animal grazers limit standing crop.

The radioisotope studies show that the algae at each station are adapted to the temperature at which they are living. This was true for algae in three kinds of habitats, epilithic, epipellic, and epiphytic, although only epilithic algae were studied in detail. The temperature optima for photosynthesis parallel the temperatures of the habitat although they were approximately 3 degrees higher. Similar results were found by Zeikus and Brock (1972) for bacteria in the Firehole River and for Brock and Brock (1968) for bacteria living at higher temperatures. To explain the fact that temperature optimum is 3°C lower than the habitat temperature, we might consider the fact that temperature optima of organisms in general are often just a few degrees below the maximum temperatures for growth. Thus a transient increase of a few degrees in a habitat, such as may occur occasionally, would push the organism to its limit and it would be eliminated from the habitat. However, if the optimum of the organism is higher than its average environmental temperature, then if a brief rise occurs, the organism will not be affected detrimentally. It may be such brief rises in temperature which eliminate organisms optimally adapted to the habitat temperature, making possible successful competition from less well adapted organisms. A consequence of this protection against harmful heating is that the organism lives most of its life at a temperature somewhat below its optimum.

There are distinct differences in algal types found in the heated and unheated parts of the river, with the main difference being a marked increase in filamentous green algae in the heated sections. Strikingly, no increase in blue-green algae was found in the heated sections, and except for station IV these algae were never dominant at any part of the river. However, the highest temperatures reached were about 27°C which are lower than those temperatures at which blue-green algae are likely to be dominant (Brock, 1970).

Although it was well established in the present work that additions of thermal water to the Firehole River result in increased algal growth rate and standing crop, it was necessary to show that it is the heating of the river which is responsible, since the thermal water is also enriched in certain important algal nutrients. Of the four most important algal nutrients, phosphate, bicarbonate, nitrate, and ammonium, only the first two increase markedly in the heated portion of the river. Of these, it seems unlikely that increased phosphate is a major factor, since even at station I the phosphate concentration is considerably higher than that considered likely to induce excessive growth of algae and other aquatic plants (Lee, 1970). Bicarbonate, measured by alkalinity titration, might play a role in promoting algal growth (Todd, 1967), although Lee (1970) has concluded that this nutrient becomes limiting only in the presence of excessive algal blooms and at station I, where alkalinity is the lowest, excessive algal growth never occurs. It might be noted that the concentrations of nutrients necessary for the development of excessive algal blooms

have been determined in more or less static situations in lakes, and in a flowing river such as the Firehole, nutrients will probably not become limiting until levels are reached much lower than those which limit algal growth in lakes. Our preliminary experiments showed no stimulation of photosynthesis by phosphate, nitrate, and ammonium at any of the stations. Bicarbonate did stimulate photosynthesis at station I, but stimulation was even greater at station VI, suggesting that bicarbonate deficiency alone cannot account for the decreased algal growth at station I. However, further studies on nutrient limitation would be desirable.

There are negligible differences in the data collected on the water chemistry in our study during the summers of 1971 and 1972 and those data collected in the summer of 1970 by Zeikus and Brock (1972). Temperature data for the three summers also agree except the temperature at station I for 1971 and 1972 runs about 3 degrees higher than that recorded during the summer of 1970. This could reflect the occurring temperature diurnal cycle which was not taken into account by Zeikus and Brock. Nevertheless the temperature data in general match closely those obtained by Armitage in 1952 and 1953 (Armitage, 1958) which would indicate that the thermal effects on the Firehole River have been stable for a relatively long period of time.

The present study further emphasizes the value of the Firehole River as a model for studies on thermal effects. As a habitat which has been subject to heating over a long period of time, the organisms present should be well-adapted to the thermal regime to which they are subjected.

Our radioisotope studies show that this is indeed the case, and that the algae maintain their temperature optima throughout the year. Thus it is the maximum temperature which the river reaches in mid-summer which controls the temperature optimum of the algae. It is likely that this maximum temperature also controls species composition and other aspects of the algal community.

Finally, it might be noted that despite the thermal additions from the geyser basins, the Firehole River is an excellent trout stream. Even in the warmest sections of the river, trout are present throughout the summer, as determined by diving with face mask and snorkel. Since trout did not exist above Firehole cascades until introduced in 1889 (Benson, Cope, and Bulkley, 1959) any adaptation to the unusual thermal regime must have occurred since then. Detailed studies on the temperature acclimation of the Firehole River trout would be of considerable interest.

ACKNOWLEDGMENTS

The technical assistance of Kathryn Boylen in some of this work is gratefully acknowledged. We thank the National Park Service for permission to carry out research in Yellowstone National Park. This work was supported by a contract from the Atomic Energy Commission (COO-2161-1 and COO-2161-2). A portion of this study was conducted while the senior^{author} was a National Research Council Postdoctoral Associate.

LITERATURE CITED

- Allen, E. T., and A. L. Day. 1935. Hot springs of the Yellowstone National Park. Carnegie Inst. Washington Pub. No. 466. Washington, D.C. 525 p.
- American Public Health Association. 1965. Standard methods for the examination of water and wastewater. 12th ed. 769 p.
- Argyle, R. 1966. Thermal dispersion in lotic waters of Yellowstone National Park. Masters Thesis. Colorado State Univ., Fort Collins. 122 p.
- Armitage, K. B. 1958. Ecology of the riffle insects of the Firehole River, Wyoming. Ecology 39: 571-580.
- Benson, N. G., O. B. Cope, and R. V. Bulkley. 1959. Fishery management studies on the Madison River system in Yellowstone National Park. Washington, U.S. Dept. of the Interior, Fish and Wildlife Service, Special Scientific Report--Fisheries, No. 307. 29 p.
- Brock, T. D. 1967. Relationship between standing crop and primary productivity along a hot spring thermal gradient. Ecology 48: 566-571.
- _____. 1970. High temperature systems. Ann. Rev. Ecol. Systematics 1: 191-220.
- _____, and M. L. Brock. 1968. Relationship between environmental temperature and optimum temperature of bacteria along a hot spring thermal gradient. J. Appl. Bacteriol. 31: 54-58.

- Eisenbud, M., and G. Gleason (ed.) 1969. Electric power and thermal discharges. Gordon and Breach, New York. 423 p.
- Fischer, W. C. 1960. Earthquake! Yellowstone's Living Geology. Yellowstone Library and Museum Association, Yellowstone Park, Wyoming. 62 p.
- Flemer, D. A., D. H. Hamilton, C. W. Keefe, and J. A. Mihursky. 1970. The effects of thermal loading and water quality on estuarine primary production. Natural Resources Institute, Univ. Maryland, Ref. No. 71-6. 210 p.
- Goldman, C. R. 1965. Micronutrient limiting factors and their detection in natural phytoplankton populations. Mem. Ist. Ital. Idrobiol. 18 Suppl.: 123-135.
- Golterman, H. L. (ed.). 1969. Methods for chemical analysis of fresh waters. Blackwell Scientific Publications, Oxford. 172 p.
- Jones, W. E. 1967. Benthos as related to water temperature, Firehole River, Wyoming. Masters thesis, Colorado State Univ., Fort Collins. 193 p.
- Krenkel, P. A., and F. L. Parker (ed.). 1969. Biological aspects of thermal pollution. Vanderbilt Univ. Press. 407 p.
- Lee, G. F. 1970. Eutrophication. Supplement to the Encyclopedia of Chemical Technology. John Wiley and Sons, New York. 39 p.
- McConnell, W. J., and W. F. Sigler. 1959. Chlorophyll and productivity in a mountain river. Limnol. Oceanogr. 4: 335-351.
- Parker, F. L., and P. A. Krenkel (ed.). 1969. Engineering aspects of thermal pollution. Vanderbilt Univ. Press. 351 p.

- Patrick, R. 1969. Some effects of temperature on freshwater algae, p.161-185.
In Krenkel, P. A. and F. L. Parker (ed.), Biological aspects of thermal pollution. Vanderbilt Univ. Press.
- Prescott, G. W. 1951. Algae of the western Great Lakes area.
Cranbrook Institution of Science. Cranbrook Press, Bloomfield Hills, Mich. 946 p.
- Roeder, T. S. 1966. Ecology of the diatom communities of the upper Madison River system, Yellowstone National Park. Ph.D. thesis, Montana State Univ., Bozeman. 85 p.
- Smith, G. W. 1950. Fresh-water algae of the United States. McGraw-Hill, New York. 719 p.
- Todd, E. H. 1967. Primary productivity of the Madison River in Yellowstone National Park, Wyoming. 120 p.
- Wetzel, R. G. 1965. Nutritional aspects of algal productivity in marl lakes with particular reference to enrichment bioassays and their interpretation. Mem. Ist. Ital. Idrobiol. 18 Suppl.: 139-157.
- Wright, J. C., and R. M. Horrall. 1967. Heat budget studies on the Madison River, Yellowstone National Park. Limnol. Oceanogr. 12: 578-583.
- _____, and I. K. Mills. 1967. Productivity studies on the Madison River, Yellowstone National Park. Limnol. Oceanogr. 12: 568-577.
- Zeikus, J. G., and T. D. Brock. 1972. Effects of thermal additions from the Yellowstone geyser basins on the bacteriology of the Firehole River. Ecology 53: 283-290.

FIGURE LEGENDS

Fig. 1. Temperature during the summer, 1971, at three stations established on the Firehole River. The station numbers are indicated by Roman numerals on the graphs.

Fig. 2. Diurnal temperature variation in the Firehole River on August 16-17, 1971.

Fig. 3. Changes in alkalinity, conductivity, and phosphate concentrations during the summer, 1971, at stations I, VI, and X.

Fig. 4. Comparison of temperature with biomass and growth rate of epilithic algal populations as a function of distance along the Firehole River.

Fig. 5. Growth rates of algal populations on clean stones placed at stations I, VI, and X.

Fig. 6. Effect of temperature on light-stimulated ^{14}C -bicarbonate incorporation for epilithic algal populations from stations I and VI. Dark control values have been subtracted.

Fig. 7. Comparison of temperature optima for photosynthesis of epilithic algae at different stations (A) with the average mid-summer temperatures of these stations (B).

Table 1. Summary of temperatures taken of the Firehole River during 1971.

Station	Site Description	Km from Station I	Jan.	May					June				July				Aug.
			27	8	14	23	29	8	15	22	29	5	13	20	27	3	11
I	Above Old Faithful	0	5.5	5.5	5.5	5.8	6.8	8.8	4.5	6.0	4.8	8.2	8.0	10.0	10.5	12.5	14.5
III	Below Morning Glory	4.8	9.0	9.0	8.9	9.2	9.2	10.5	7.0	8.8	6.8	11.6	12.5	13.8	15.0	17.5	19.8
IV	Below Biscuit Basin	6.3	11.5	11.0	11.2	11.2	10.8	11.5	8.0	10.2	8.6	14.0	15.5	16.0	18.0	21.2	22.0
V	Below Midway	10.3	14.5	13.0	12.8	13.0	13.0	14.0	11.0	13.8	12.0	18.0	17.5	19.0	20.0	23.8	25.0
VI	Above Ojo Caliente	14.0	18.5	15.0	-	15.2	14.5	16.5	14.0	16.8	14.8	20.5	24.5	24.0	24.0	26.8	27.0
VII	Above Nez Perce	16.7	-	-	-	-	-	15.0	13.5	16.2	14.0	19.8	20.5	22.0	24.0	27.0	26.5
VIII	Firehole Canyon	19.8	12.0	10.8	11.0	11.4	9.1	15.0	14.0	18.4	11.8	16.5	23.2	21.0	23.0	25.0	25.0
IX	Gibbon River	23.5	7.5	8.8	8.8	10.0	8.8	14.2	15.0	20.5	13.2	18.5	21.5	-	20.5	-	-
X	Madison River	34.0	-	14.8	10.8	10.8	11.0	14.3	15.0	19.8	13.8	14.2	22.0	20.0	20.5	22.0	22.0

Table 2. Temperature and water analysis of Firehole River at different sampling stations*

Station	Temperature °C	pH	Alkalinity mg/l CaCO_3	Conductivity $\mu\text{mhos/cm}$	Phosphate $\mu\text{g/l}$	Nitrate $\mu\text{g/l}$	Chloride mg/l	Ammonium $\mu\text{g/l}$
I	12	7.4	15	65	50	240	7	70
III	17	7.6	32	160	125	250	22	60
IV	20	7.7	46	220	200	270	25	50
V	23	8.1	64	285	295	210	34	55
VI	26	8.3	90	420	485	190	57	45
VII	25	8.3	88	410	485	210	53	50
VIII	24	8.3	84	385	425	140	50	45
X	21	7.8	88	360	345	190	41	60

*Data are arithmetic averages for the period July 20-August 17, 1971 when water depth was at a minimum.

Fig. 4

Fig. 4

Figure 5