Thermal Pollution of a Small River by a Large University: Bacteriological Studies

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Abstract

The Jordan River, which flows through the Bloomington campus of Indiana University, arises on the campus as a series of small cold-water springs. Temperature measurements indicate that one effect of the university on the river is an increased water temperature. The temperature optima of bacteria at selected stations on the river and in several cold-water springs away from the campus have been measured using an *in situ* radioisotope technique. It was found that the temperature optima in general parallel the temperature of the habitat. Studies also showed that *Thermus aquaticus*, a non-spore-forming extreme thermophile, was present in heated but not in non-heated water and might be used as an indicator of thermal pollution.

Introduction

The Jordan River, which flows through the Indiana University campus at Bloomington, arises on the campus as a series of small cold-water springs. This small river, with flow rate less than 1 cu ft/sec, is now being influenced by the university complex, and temperature measurements provide an excellent picture of how such a large building complex, in no significant way different from any other urban development, has increased the water temperature of the river. This has happened even though the university does not intentionally affect the river. The thermal additions arise either from unobserved leaks in steam lines, from condensate of air conditioning units, or from cooling units of distilled water systems.

In the present paper we provide data on the extent of the thermal effect and present studies on the resident bacterial populations found in normal and heated water.

Methods

Temperature was measured with a Yellow Springs Instrument Co. thermistor. The temperature optima of the resident bacteria were determined using the cover slip technique of Bott and Brock (2), using C^{14}-acetate. The presence and amount of *Thermus aquaticus* in water samples was determined using a nine-tube Most Probable Number method (1) with three tubes at each dilution of the water sample. Incubation was at 70°C in the medium of Brock and Freeze (5) and all tubes showing turbidity after 1, 2 and 3 days were noted. On the third day of incubation, all tubes showing turbidity were streaked on agar medium and after incubation at 70°C the presence of typical yellow pigmented colonies noted. Yellow colonies were examined microscopically for the presence or absence of spores and for the typical *T. aquaticus* morphology.

Results

Figure 1 shows the general geography of the area and the location of stations where temperature readings were made. Temperatures were taken at weekly or monthly intervals, depending on the season of the year. Temperatures at several control areas were also taken. Ellettsville Spring is a typical cold-water spring about 10 miles from the campus and remains constant in temperature at about 12°C year around. Jackson's Creek is a small creek arising in the same geological formation as the Jordan River but flowing in a different direction. Its temperature varies throughout the year in parallel with variations in air temperature. Figure 2 provides temperature data at selected stations for about 1 year; detailed data at all stations can be obtained from the authors. Station 15 on the Jordan River is sufficiently away from sources of heat that its temperature also varies with air temperature. The other stations on the Jordan River show heating effects of various magnitude. Station 30b is the most dramatic, its temperature ranging around 48-56°C throughout the year. This station is a small side branch immediately adjacent to one of the large steam pipes. Station 10 is the effluent from the air conditioner for the large university auditorium. Although it only flows in the summer, when flowing it averages 36°C. Station 16 is the branch arising as five springs beneath Jordan Hall. The exact source of the heat for this station is not known but we do know that the springs themselves are warm as they come out of the ground beneath the building.

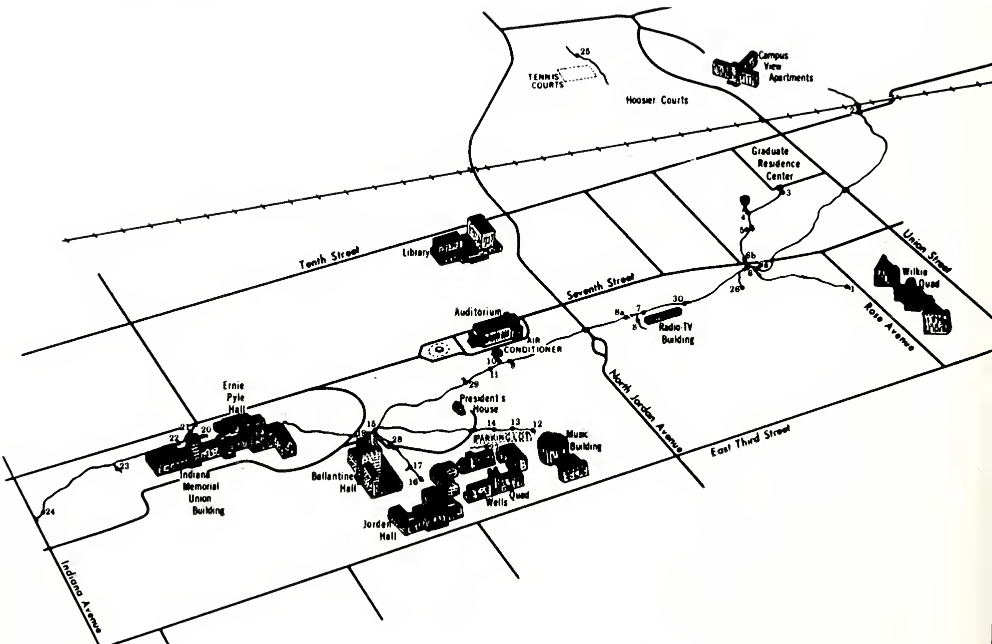

FIGURE 1. *Map of Jordan River through the Indiana University campus, giving locations of stations where temperatures were measured. Not all buildings are shown.*

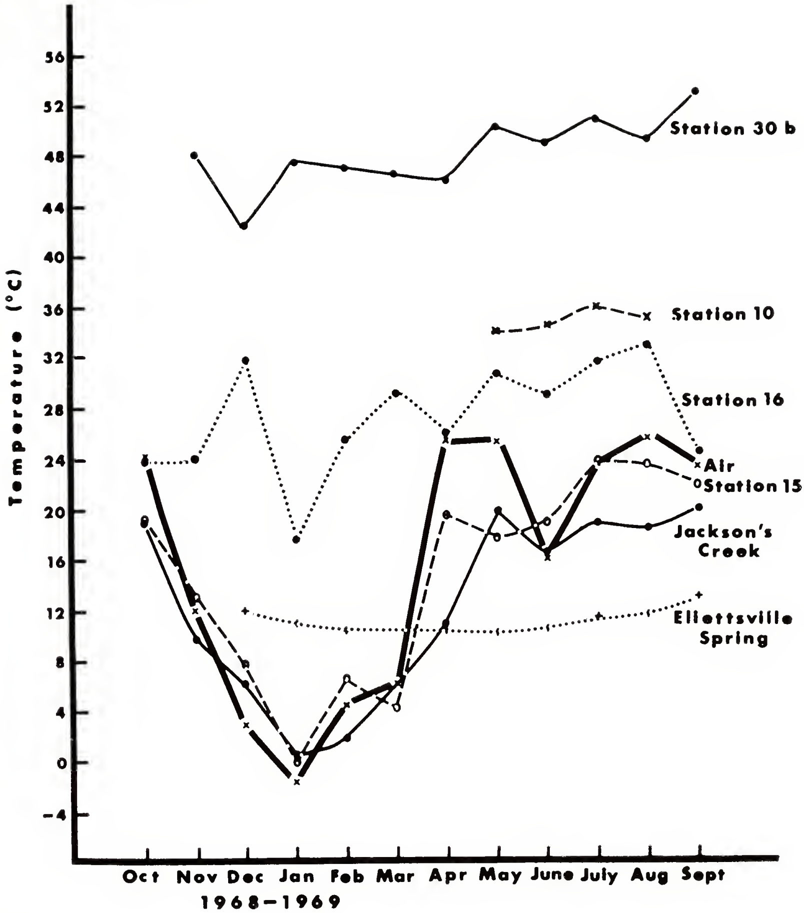

FIGURE 2. *Annual variation in temperature at selected stations. Those stations not otherwise specified are in the Jordan River on the Indiana University campus.*

After we had identified a series of habitats of differing temperature characteristics we determined the temperature optima of the resident bacteria using the cover slip-isotope technique described by Bott and Brock (2). It was assumed that the bacteria which grew on submerged cover slips were representative of those living attached to natural substrates in the water. By using colonized cover slips we were certain that the organisms we were dealing with had developed at known temperatures. Temperature optima were then determined by measuring the rate of C^{14}-acetate incorporation of replicate cover slips incubated with the isotope at different temperatures. Note that cover slips were incubated with isotope only for an hour and that they were used without pretreatment and within a few hours (at most) after removal from the habitat. At no time before the isotope experiment was begun were the cover slips allowed to reach temperatures significantly different from those in the natural environment.

Figure 3 presents representative data. The stations studied include those given in Figure 2 which should be studied in comparison.

The temperature optima in general parallel the temperatures of the natural habitats, the warmer habitats having higher temperature optima. These results confirm other studies carried out in Yellowstone National Park (4). However, the population developing in Ellettsville Spring, where the temperature is 12°C year around, has an optimum higher than 12°C.

Recently we have described a new genus and species of bacteria, *Thermus aquaticus*, representatives of which are widespread in thermal environments, both natural and man-made (5). *Thermus aquaticus* is a non-spore-forming yellow pigmented bacterium which grows optimally at about 70°C, but grows also at 50-55°C. Since it was found in hot tap water and in steam lines, it seemed possible that it could be used as an indicator of thermal pollution. The procedure, described in Methods, was based on sanitary bacteriological water analysis. Representative data are given in Table 1. Note that these studies were done in summer when the unpolluted streams are fairly warm, although the springs of course remain cool. The results show that *T. aquaticus* was not found in the cool springs or a normal stream, but was found in the thermally polluted Jordan River. The counts at Station 30b, temperature 56°C, were quite high.

Discussion

These data provide a quantitative measure of the extent to which Indiana University causes heating of the Jordan River. Our main purpose, however, was to use the river as a convenient habitat for studies on bacteriological aspects of thermal pollution. The use of the cover slip-isotope technique permits *in situ* study of the temperature optima of bacterial populations which have developed under known temperature conditions. It is clear that the bacteria developing in the warmer waters have temperature optima which parallel the temperatures of their environments. These observations suggest that in the warmer waters, once a stable temperature has been maintained at a location for some time, if the water should become either cooler or warmer, bacterial metabolism should slow down. Thus one cannot assume that a further warming of the water will lead to an immediate increase in bacterial metabolism. However, the temperature optimum of the bacteria living in the cold Ellettsville Spring is considerably higher than the temperature of the habitat. Unpublished studies in some other cold springs in southern Indiana by Fred Passman also showed similar results. Thus bacteria living at low temperatures are not optimally adapted to their environment and will probably begin to grow and metabolize faster immediately upon being warmed. Thus the initial effect of thermal pollution in cold springs should be to increase bacterial action.

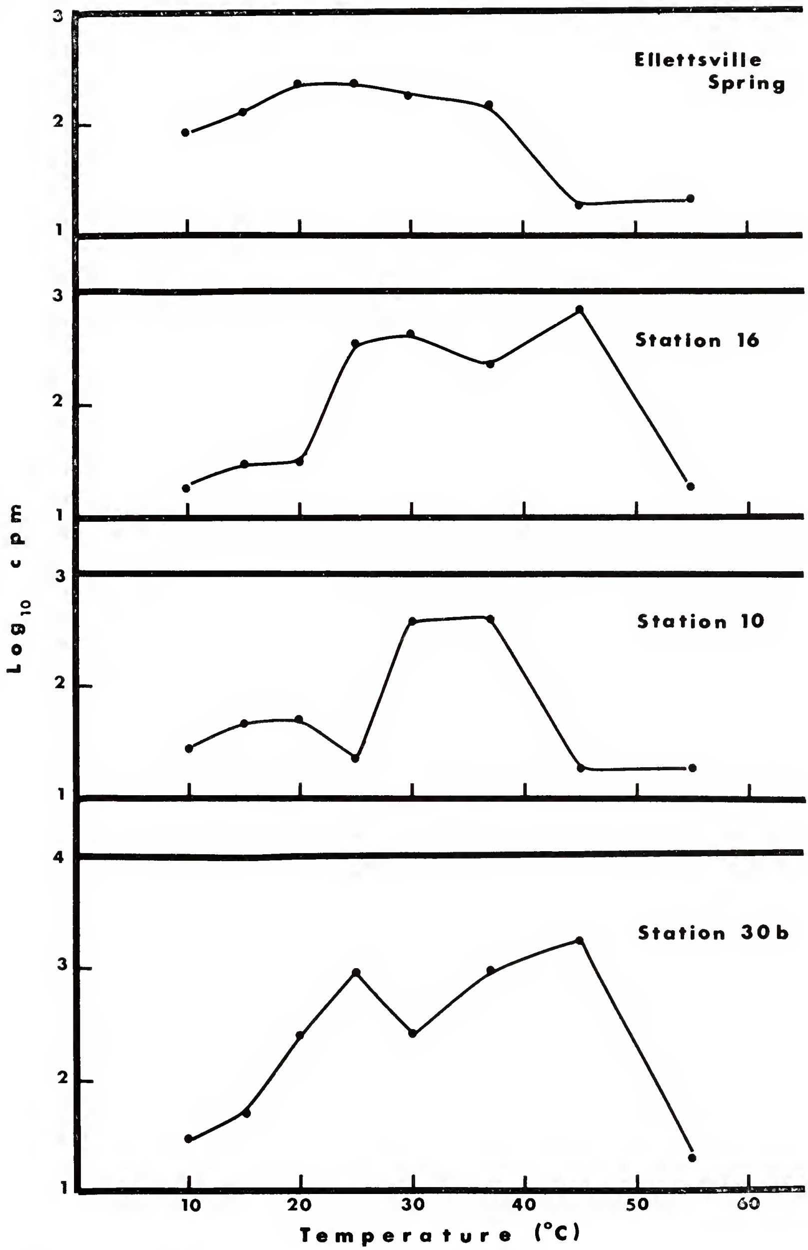

FIGURE 3. *Temperature optima of microflora developing on substrates immersed at the stated locations. For temperatures of the stations through the year, see Figure 2.*

TABLE 1. Thermus aquaticus *as an indicator of thermal pollution.*

Location	Date	Temperature °C	MPN Index/100 ml
Cold-water Springs			
Farm Spring	7/ 2/69	9.0	N.D.
Ellettsville Spring	7/ 2/69	11.5	N.D.
Unpolluted Stream			
Bean Blossom Creek	8/19/69	24.0	N.D.
Jordan River			
Below station 12	7/16/69	34.0	20
Station 16		14-42	*
Station 30b	8/19/69	56.0	15,000

MPN index calculated as described in (1).
N.D. None detected.
* Non-pigmented *T. aquaticus* detected by Ramaley and Hixson (6).

The Jordan River is a convenient study area as it is readily accessible to our laboratory and long term changes in thermal characteristics can be followed. In addition to the present work, this river has been used in studies on the growth of *Sphaerotilus,* a filamentous bacterium often associated with organic pollution (3).

Acknowledgments

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