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PROJECT COMPLETION REPORT

PLANKTON HETEROTROPHY IN A NORTH CAROLINA ESTUARY

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Abstract

The heterotrophic ability of the bacteria of the plankton was studied with a technique utilizing the uptake of radioactive organic compounds. Although the method was developed in freshwater, it proved to be adaptable to estuarine conditions. In this project, which was the first phase of a larger study of the role of bacteria in the aquatic ecosystem, it was found that glucose and acetate were taken up by the bacterial uptake systems and that the uptake rate could be analyzed by the equations of enzyme kinetics. The maximum velocity of uptake appeared to be a good indication of relative heterotrophic activity and reached a peak during the summer in the Pamlico River estuary. This parameter was also a rapid and sensitive indicator of eutrophication as peaks of activity found in a 30 km survey were correlated with domestic wastes and with effluent from phosphate mining activity.

Key words: heterotrophic bacteria*, plankton, glucose, acetate, uptake rate, indicator*, eutrophication*, domestic wastes, phosphate mining effluent*.

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Objectives, Results, and Conclusions

The work covered in this report represents the first part of a long-term study designed to elucidate the role of the aquatic bacteria in the metabolism of natural waters. Some of the topics that will be investigated in this long-term study include: the nature and source of some organic compounds used by aquatic bacteria; the rate of use of these compounds; the use of heterotrophic activity as an index of eutrophication. All of this work is based upon a recently developed technique (Hobbie and Wright 1965, Wright and Hobbie 1966) utilizing a measurement of the uptake of organic radioisotopes by the natural bacteria of freshwaters.

The objectives of this first phase of the project were: to carry out experiments to adapt the heterotrophic uptake technique to estuarine conditions; to identify some of the organic substrates being used by the heterotrophic bacteria; and to measure the rate of uptake of some of these compounds and the seasonal and spatial changes of this rate in the Pamlico River estuary complex.

It was found that the estuarine bacteria responded in the same way as the freshwater forms and that the uptake of organic radioisotopes followed Michaelis-Menten kinetics. Heterotrophic activity in South Creek was low in the winter, spring, and fall and up to 20 times higher in the summer. Several samplings of the spatial distribution of V showed that the highest activities were associated with the eutrophication entering the river from a town (Aurora, N. C.) and from phosphate mining effluent. The method holds promise as a fast and sensitive measure of eutrophication.

Background of Procedures Used

The importance of heterotrophic bacteria in aquatic ecosystems has long been debated but there are few facts available upon which to base reasonable conclusions. Certainly there is a tremendous variety of organic compounds dissolved in freshwater (reviewed by Vallentyne 1957) and in seawater (reviewed by

Duursma 1965) and it is equally certain that heterotrophic bacteria are living upon these compounds for a variety of bacteria may be cultured (Wood 1963). However an ecologist is interested in rates of production of bacterial biomass from this organic matter as well as rates of formation of the organic compounds. In the summary of a 1963 meeting on aquatic bacteriology, Heukelekian and Dondero (1964) suggested that radioisotope techniques might profitably be brought to bear on the problem of rate measurement in some of the bacterial processes, as the traditional methods of plate culture or of direct counts of the bacteria gave little usable information. Some limnologists had already begun experiments where organic isotopes, such as glucose-C^{14}, were added to flasks of lake water (Saunders 1958, Rodhe 1962), but their data could not be interpreted as both bacteria and algae were taking up the compounds and as the extremely artificial experimental conditions could not be related to the natural condition. The breakthrough came with the work of Parsons and Strickland (1962) who showed that the uptake of these radioisotopes could be described by the Michaelis-Menten equations. This finding brought some order to a chaotic situation and allowed extrapolations from the laboratory results to the natural situation.

The techniques of Parsons and Strickland (1962) were drastically modified by Wright and Hobbie (1965) to eliminate interference by algal uptake of the organic isotopes. Subsequently, it was found that the algae were not important in natural waters as heterotrophs (Wright and Hobbie 1966) and that, by working at added substrate concentrations close to the natural substrate concentration (e.g. a few µg/l), only the uptake by bacteria was measured. By analyzing the uptake of radioisotope by the aquatic bacteria at a number of substrate concentrations, the following information could be obtained: the maximum velocity of uptake of the substrate by the bacterial population, a measure of relative heterotrophy; the maximum amount of substrate present in nature; the time required for the bacteria to completely remove the substrate (turnover time of the substrate).

Procedures Used

The techniques used were those outlined in Wright and Hobbie (1966) with a number of modifications for estuarine work.

Samples of estuary water were collected in plastic bottles (2 liter) and returned to the laboratory within 2 hours. From each sample, a series of 25 ml subsamples were poured into 125 ml glass stoppered Pyrex bottles. A small amount of radioactive substrate was next added to each bottle. With glucose, for example, the series of substrate concentrations was .003, .006, .009, and .012 mg glucose/liter. One additional sample with .012 mg/l was killed immediately with acid Lugol's solution (2 drops) as a blank. The other samples were then incubated in a running sea water bath for various lengths of time depending upon the temperature. After the incubation and killing of the samples, the organisms were filtered onto 0.5 μ Millipore filters and the activity of the organisms measured in a G-M thin window counter.

Calculations followed Wright and Hobbie (1966) with the quantity $(S + A)/v$ as the computed value. Here, S equaled the amount of natural substrate present in the water (mg/l), A was the substrate added in the experiment, and v was the natural velocity of uptake of the substrate by the bacteria. It was shown in the above mentioned paper that this value could be obtained even though the S and v were unknown. A plot of $(S+A)/v$ versus A (abscissa) yielded the maximum velocity of uptake (V) as the reciprocal of the slope of the line, the turnover time of the substrate (T) as the ordinate intercept, and $-(K + S)$ as the abscissa intercept. K is a constant resembling the Michaelis constant.

Results and Conclusions

One of the first problems in transferring this method from a freshwater to a marine environment is that of the incubation time. This should be short enough so that only a small percentage of the available substrate is taken up yet long enough so that a few thousand counts per minute (CPM) are obtained

on the filter. This time could vary from ½ to 12 hours depending upon the temperature and the activity of the bacteria. The incubation time should be changed to fit the changing temperatures and activities throughout the year.

Measurements of the uptake of glucose-C^{14} over time (Fig. 1) showed that there was a linear uptake for several hours followed by a decrease in the rate of uptake. This was true at all substrate concentrations. Therefore, at this temperature (6°C) an incubation time of 2 hours appears to be ideal. Other measurements at various temperatures gave the following data: 30°C, 1.5 hr; 15°C, 2 hr.

When plotted as (S + A)/v versus V, a straight line results (Fig. 2) showing that the uptake of the radioisotopes followed Michaelis-Menten kinetics. In this example, the V is 1.6×10^{-4} mg/l/hr, the (K + S) is .002 mg/l, and the T is 1.5 hr. This indicates that the maximum rate of uptake of this substrate by the bacteria is 1.6×10^{-4} mg/l/hr., the maximum amount of substrate present is .002 mg glucose/l, and that the total glucose present is being completely removed every 1.5 hours. The V is a good indicator of the relative heterotrophic activity and, as such, has a direct relationship to the trophic level of the water body. For example, in a series of Swedish lakes ranging from oligotrophic to strongly polluted, there was a range of 5 orders of magnitude in the V (Wright and Hobbie 1966).

The seasonal range in V will depend upon the general level of primary productivity, the degree of pollution, and the temperature of the water. Some data for glucose V at the Pamlico Marine Laboratory pier are given in Table 1. It is obvious that the low V values were found in the spring and fall and the

Table 1. 1966 vlaues of V in South Creek, at the P.M.L. pier.

Date	V ($\times 10^{-4}$)	Date	V ($\times 10^{-4}$)
16 Jan.	0.8	18 July	20.1
7 May	4.4	28 July	2.5
26 May	5.0	24 Aug.	25.0
8 June	15.6	24 Sept.	12.4
22 June	9.0	23 Oct.	5.5

Fig. 2

Aurora, N. C.
28 July 1966
Buoy #14
South Creek
29°C

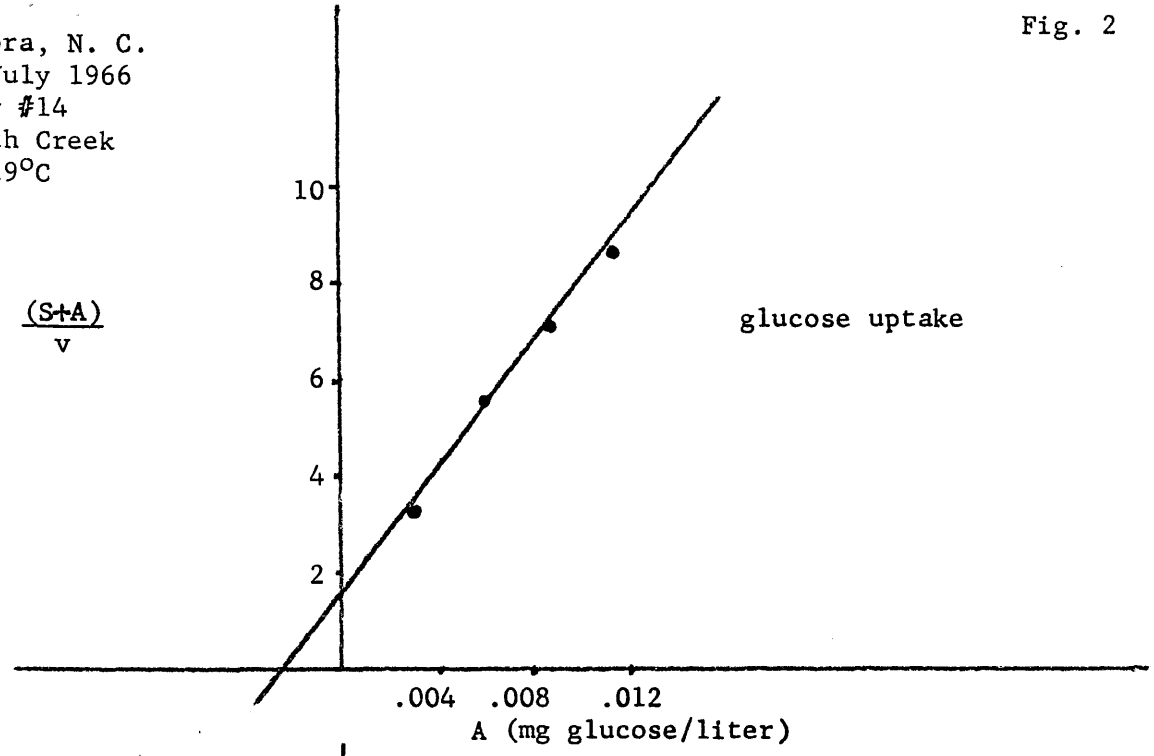

Fig. 1

Aurora, N. C.
16 January 1966
glucose

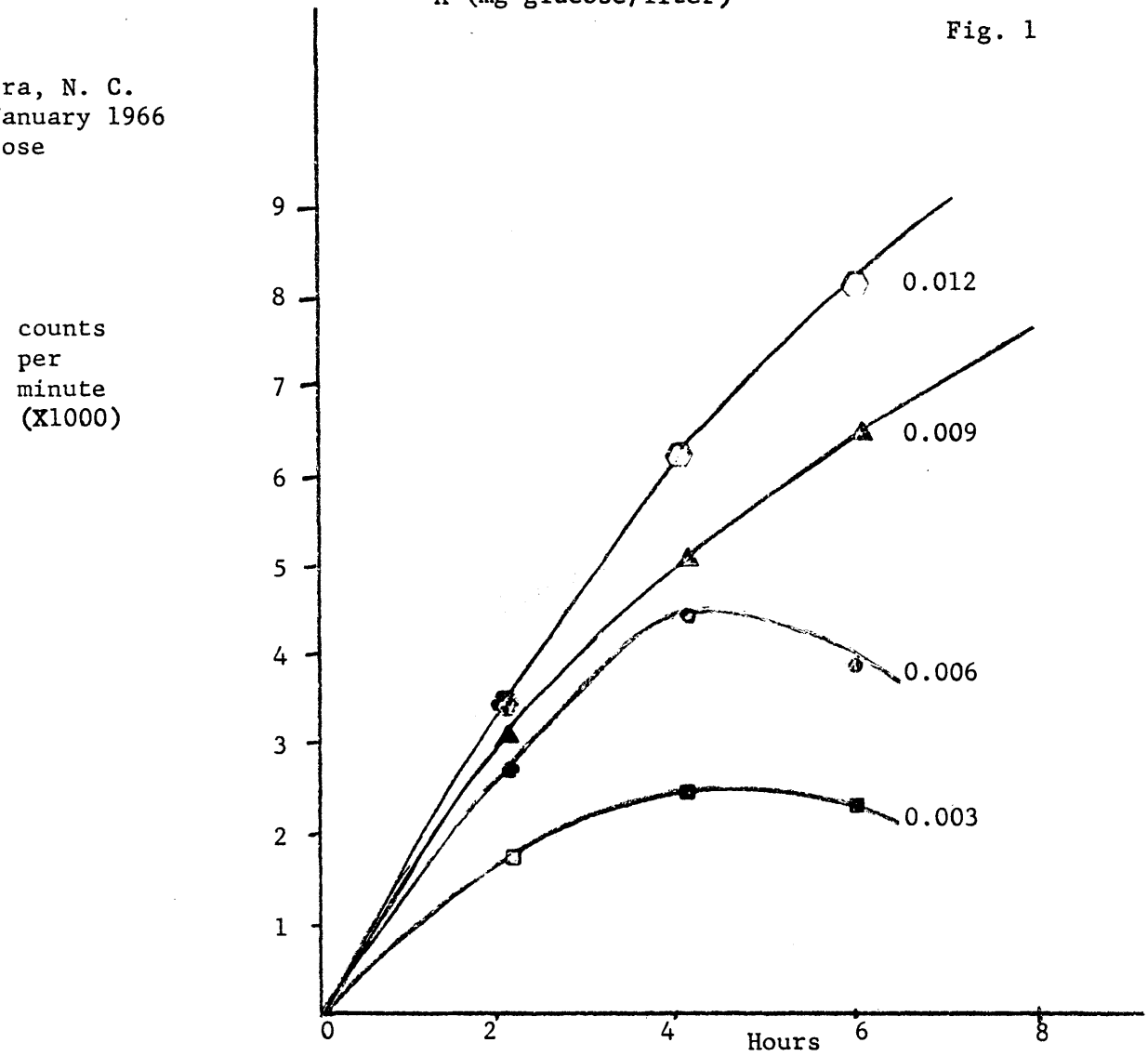

Fig. 1. Uptake of glucose-C^{14} over time. Aurora, N. C., P.M.L. pier.

Fig. 2. Uptake of glucose-C^{14} at various substrate concentrations. Plotted as (S + A)/v versus A.

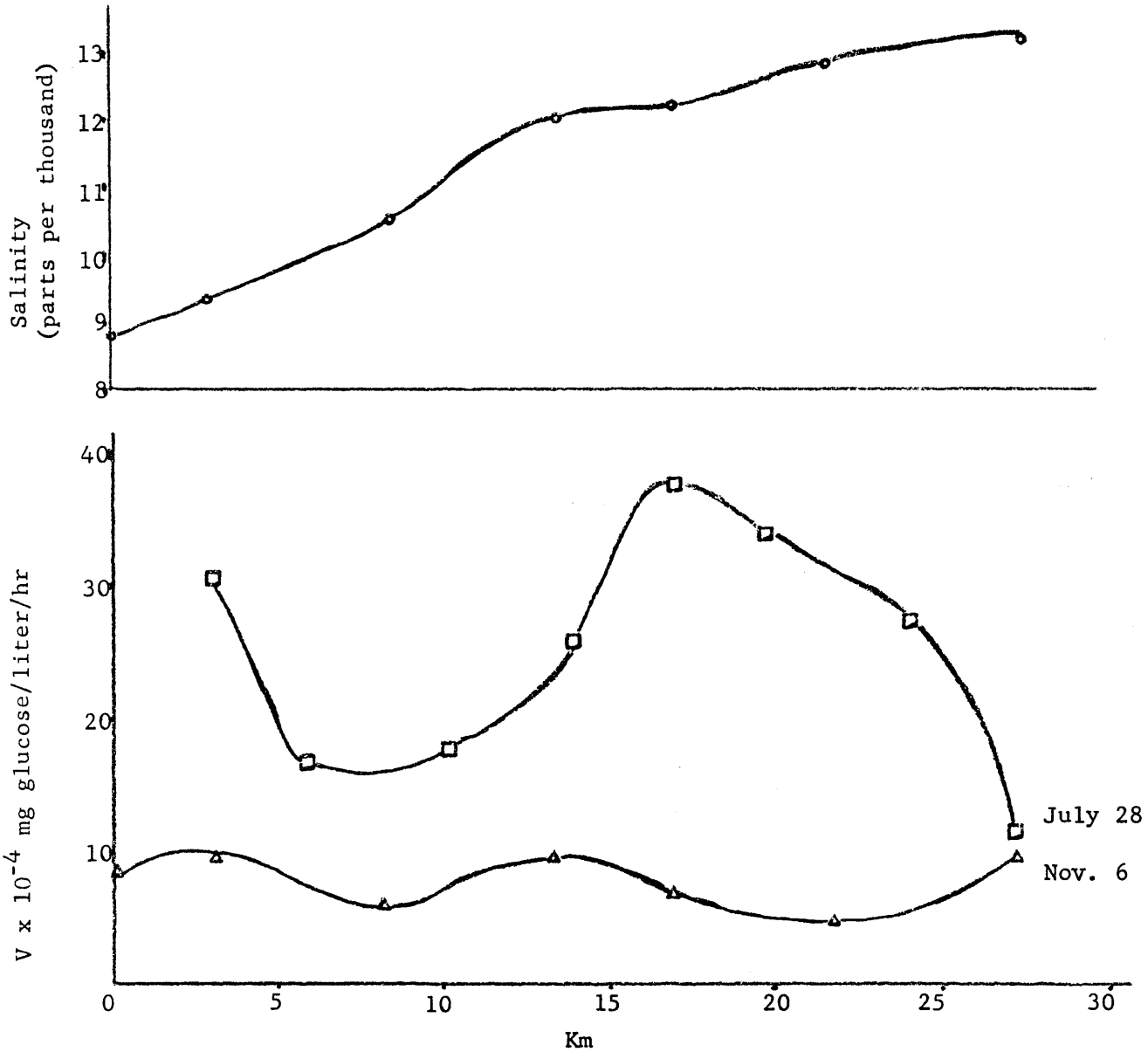

Figure 3. Variations of maximum velocity of uptake (V) and of salinity in the South Creek - Pamlico River complex.

high values in the summer. The low values noted in June and July need further data for explanation.

The spatial change in V is chiefly a function of the degree of eutrophication or of oligotrophy-heterotrophy in the body of water. Because of this, the technique could be important in future work on eutrophication. Some preliminary runs were made in the South Creek-Pamlico River estuary complex to test the spatial distribution of V. South Creek drains swamp and low-lying land and the water is stained with humic material. The town of Aurora lies at 2.5 km on Figure 3, and the Pamlico Marine Laboratory at 14 km. The Pamlico River is reached at about 24 km. Salinities range from 8 parts per thousand at Aurora to 13 parts in the River. Effluent from the holding pond of the Texas Gulf Sulfur Co. phosphate mining operation enters the estuary at 17 km, but flows upstresm along the edge of the river until about 10 km. The two peaks of heterotrophic activity are thus explained by sewage form the town of Aurora and by the T.G.S. effluent water. Although the method appears to be a very sensitive one, comparisons must be made with other measurements of eutrophication before a real evaluation will be possible.

Several other substrates were tested via uptake by bacteria to determine which might be important in the estuary. Those tested were acetate and glycine. The uptake of the acetate was very similar to that of glucose, with a V of 3.61×10^{-3} mg/l/hr, a (K + S) of 0.011 mg/l and a τ of 4 hours. The glycine showed a very low uptake that did not show Michaelis-Menten kinetics. Glycine is a poor source of food for bacteria, so other amino acids may well give better results.

Other experiments with a vertical series of samples in the water column revealed peaks of heterotrophic activity at the top and bottom of the water column, but better sampling methods are needed. It appears that there were only minor changes of V with depth, but that the surface decimeter should be avoided if a spatial study is planned.

In conclusion, this particular method can be adapted to estuarine conditions and the estuarine bacteria respond in the same way as the freshwater forms. The estuarine environment is quite rich, so a two hour maximum incubation was used. There were 20 fold variations in the V from January through October and the highest values of bacteria heterotrophic activity were found in later August. In addition, there was a very interesting spatial distribution over distance in South Creek and the Pamlico River. One peak of heterotrophic activity was noted close to the town of Aurora and another peak could be related to the Texas Gulf Sulfur mining effluent. The method is a very sensitive measure of eutrophication, but more work is needed using other indexes of eutrophication for comparison.

Achievement of Objectives

The general objectives of this project were achieved in that the techniques were modified so that they could be used in the estuary, and that the temporal and spatial variations of V were measured. Only 3 substrates were tested, however, and so the objectives were not achieved in this particular.

Publications from this Project

As this project was really the start of a larger project, no publications have resulted. However, some of this data will be incorporated into later papers and will be credited to this project.

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