THE RELATIONSHIP OF TURBIDITY AND ORGANIC MATTER TO BACTERIAL PRODUCTIVITY AND BIOMASS IN A SMALL TEXAS LAKE

A Thesis Submitted to the Faculty of
Baylor University
in Partial Fulfillment of the
Requirements for the Degree
of
Master of Science

by
Laura Dávalos

Waco, Texas
December, 1986

ABSTRACT

The relationship between water turbidity and bacterial cell synthesis, specific production and bacterial productivity was investigated in a small Texas reservoir. Sampling dates were selected to include a variety of water turbidities. Bacterial cell synthesis calculated from rates of ^{3}H-thymidine incorporation ranged from $1.97X10^{7}$ to $4.79X10^{7}$ cell l^{-1} h^{-1}. Specific production ranged from 0.006 to 0.03 ug C ug C^{-1} l^{-1} h^{-1}. Bacterial production varied between 0.23 and 0.83 ug C l^{-1} h^{-1}.

Multiple regression analysis showed that turbidity explained 63.6% of the variation in cell synthesis. When combining turbidity with the biomass of the particle-attached bacteria 82% of the changes were explained and 95.6% were explained when combining turbidity, attached cell biomass and total organic carbon. Changes in specific production were determined primarily by the turbidity (r=.76), combining turbidity with dissolved organic carbon the variation explained was 90.3%. The absolute bacterial production changes were not explained by any of the environmental variables measured during the study.

RESUMEN

La relación entre la turbidez del agua y la síntesis de células bacterianas, la producción específica y la productividad bacteriana se investigo en una pequeña represa texana. Los días de muestreo se seleccionaron de manera que se incluyera variación en la turbidez del agua.

La síntesis bacteriana, calculada a partir de la incorporación de timidina tritiada, varió de $1.97X10^{7}$ a $4.79X10^{7}$ celulas l^{-1} h^{-1}. La producción específica varió de 0.006 a 0.03 ug C ug C^{-1} l^{-1} h^{-1}. La produccion bacteriana varió entre 0.23 y 0.83 ug C l^{-1} h^{-1}.

El análisis de regresión múltiple indicó que la turbidez explica 63.6% de la variación en la síntesis de células y la turbidez en combinación con la biomasa de las bacterias adheridas a partículas explica 82% de la variación y el 95.6% de los cambios es explicado cuando se combinan la turbidez, la biomasa de las celulas adheridas y el carbón orgánico total. Los cambios en la producción específica fueron explicados primeramente por la turbidez (r^2=.76), y combinando turbidez y carbon orgánico disuelto la variación explicada es el 90.3%. Los cambios en la productividad

bacteriana absoluta no son explicados por ninguna de las variables ambientales medidas durante el estudio.

Table of Contents

List of Figures

List of Tables

ACKNOWLEDGMENTS

This investigation was done thanks to Baylor University's Third World Scholarship program.

I would like to thank my professor Dr. Owen T. Lind, for his admirable patience and invaluable help, and I thank Winnie Lind for her support. Also I want to thank Dr. Darrell S. Vodopich for his help in the preparation of this manuscript.

Finally I want to thank my parents, Olivia and Manuel Davalos, for their love and support.

AGRADECIMIENTOS

Este trabajo se realizó gracias al programa de becas que sostiene la Universidad de Baylor para el tercer mundo.

Quiero expresar mi más sincero agradecimiento para mi maestro el Dr. Owen T. Lind, por su admirable paciencia e invaluable ayuda, así como a Winnie Lind por su apoyo. Tambien quiero agradecer al Dr. Darrell S. Vodopich por su incansable ayuda en la preparación de este manuscrito.

Por último agradezco a mis padres, Olivia y Manuel Dávalos, por su comprensión y constante apoyo.

INTRODUCTION

The object of my study was to determine the relation of environmental factors such as temperature, turbidity, phytoplankton density, and dissolved and total organic carbon to the bacterial productivity of an aquatic ecosystem. My principal hypothesis was that the clay (turbidity) of the water increases the bacterial production.

The importance of bacteria as mineralizers in most ecosystems is unquestioned. But the quantitative importance of bacteria as biomass producers in aquatic ecosystems only recently has been appreciated. In the past decade, marine ecologists have become aware of the role of bacterial productivity in the detrital food chain, but little attention has been given to bacterial productivity in freshwater.

Traditionally phytoplankton has been considered the most important source of particulate organic matter for herbivores and detritivores. Bacteria also can constitute an abundant food resource for fine-particle feeding organisms. Bacteria can comprise 10 to 83% of the total suspended biomass in aquatic environments (Palumbo, et al., 1984).

Bacteria may live in the water free and motile or growing on solid particles, mainly detritus. Both types, free and attached, occur in the planktonic community. The most dense populations of bacteria occur at the surface of the water (Wetzel, 1983; Rheinheimer, 1985).

The sources of organic matter for heterotrophic bacteria are diverse. The presence of algae promotes the growth of bacterial populations (Grant and Long, 1981; Van Es and Meyer-Riel, 1982; Currie and Kalff, 1984; Gude et al., 1985) either because of direct effects of exudation by the algae or because zooplankton only partially digests the algal food, and the rich fecal material is used rapidly by bacteria. Usually the bacterial maximum density is at the same depth as the phytoplankton (Campbell, 1983). Also another important source of organic matter, the allochthonous is the organic matter of terrestrial origin transported by runoff water.

The organic matter in aquatic ecosystems is in dissolved or particulate form. Particulate forms are colonized by bacteria. Inorganic particles adsorb dissolved organic matter present in the water in great dilution. The bacteria can find a more favorable environment here than in the surrounding water (Pomeroy, 1984; Rheinheimer, 1985).

Bacteria transform dissolved organic carbon to particulate organic carbon, making this resource available to higher trophic levels. Both free living and attached bacteria are grazed by a variety of organisms including some crustaceans, insect larvae, and flagellates that are important as food for larger fauna (Paerl, 1978).

The small size of bacteria results in intimate contact with their environment, therefore they are sensitive to changes in the environment. Physical and chemical factors influence the metabolic activity, the growth, and the composition of the microbial populations, thus having a direct effect on the food chain. The assessment of the relative importance of the factors that determine bacterial production of particulate matter is essential to a better understanding of the flow of energy and carbon through the aquatic food web.

LITERATURE REVIEW

Bacteria and the Aquatic Food Web: Conversion of Organic Matter

Bacteria play an important role as direct and indirect producers of particulate organic matter in aquatic ecosystems. As such they are essential components of nearly all ecosystems.

The role of bacteria as indirect producers is in promoting primary production. They transform natural organic compounds into the inorganic components from which they originated. The nutrients are returned to the cycle of matter and allow new plant growth-- new organic matter production. The remineralization of organic substrates in the turnover of matter in water is mainly the function of bacteria and fungi (Rheinheimer, 1985).

Detritus in aquatic ecosystems constitutes a large organic reserve of energy in both dissolved and particulate forms. In these ecosystems the living biota constitutes a small portion of the total organic matter (Wetzel, 1983; Pomeroy, 1984). Organic matter can be synthesized within the water by the primary producers (autochthonous), or imported to the system by runoff and wind action

(allochthonous).

Allochthonous organic matter coming from the watershed is mostly dissolved (Wetzel, 1983). The major source of autochthonous organic matter is the phytoplankton and littoral flora photosynthetic activity. Both dissolved and particulate forms can be transformed (degraded) in the water column or settle on the bottom where transformations also occur (Wetzel, 1983). Independent of where the transformations take place, these processes allow the synthesis of new organic compounds.

The bacterial role as direct organic matter producers is due to the uptake and conversion of dissolved organic matter into particulate material. Bacteria take up dissolved organic substances which are released into the water by the primary producers, released by zooplankton, released by the microbial food web, or are carried in by runoff from the watershed (Pomeroy, 1984). These organic matter sources are usually rapidly converted into particulate material, and in this way, the dissolved organic matter will be back into the food chain.

When algal cells grow some portion of their photosynthesis is released as dissolved material. This release may be due to autolysis, mechanical breakage by zooplankton, or to active excretion of metabolites (Cole et al., 1982; Wetzel, 1983). The quantity and quality of the

released dissolved organic matter by phytoplankton depends upon the species and their physiological state (Wetzel, 1983).

Bell and Mitchell (1972) introduced the term "phycosphere" to denote the zone surrounding phytoplankton that includes both inhibitory and stimulatory effects. Living phytoplankton repel bacteria, but as phytoplankton weaken and die they are invaded and converted into detritus and bacterial biomass (Fuhrman et al., 1980).

A close coupling between bacterial and algal production might be hypothesized for the plankton. Fuhrman et al. (1980) found that the bacterioplankton and phytoplankton tend to be abundant in the same places. The extracellular algal products are taken up almost instantaneously by bacteria (Larsson and Hagstrom, 1979).

There is a stimulation of bacterial activity in response to algal blooms (Bell and Sakshaug, 1980). Maximum bacterial densities show high correlations with chlorophyll *a* maxima (Fuhrman et al., 1980; Gude et al., 1985; Lovell and Konopka, 1985). Increases in bacterial numbers can occur also after the spring blooms, presumably in this case, due to organic matter from decaying cells instead of algal exudates (Blaauboer, 1982; Brock and Clyne, 1984).

Bacterial growth rates usually increase in response to increasing primary production which supplies a continuous source of dissolved substances (Lancelot and Billen, 1984; Murray and Hodson, 1985; Gude et al., 1985; Lovell and Konopka, 1985). However, Fuhrman et al. (1980) did not find a correlation between bacterioplankton and primary production; they explain this by high grazing action upon the phytoplankton population so as to limit exudate production.

Of the total carbon fixation by phytoplankton, values of 5, 30 and up to 95% release as exudates have been found (Fogg, 1971; Coveney, 1982). Cole et al. (1982) found up to 40% of the gross algal exudate was consumed daily by bacteria in Mirror Lake; 21% in Kiel Fjord (Wolter, 1982); 10 to 80% in Lake Norrviken (Bell et al., 1983); 20% in Lake Mendota (Brock and Clyne, 1984); 32 to 95% in two Swedish lakes (Coveney, 1982).

Heterotrophic utilization of algal products is a function of both the activity of heterotrophs and of the suitability of the excreted material. The phytoplankton excretion, which apparently is not an important loss for the algae, may be an important dissolved carbon source for planktonic bacteria if it is a more suitable substrate than the background dissolved organic matter (Coveney, 1982). In this way dissolved organic matter released will be rapidly

returned to the food chain even in the presence of large amounts of detrital material.

Another source of dissolved organic matter is that released by the zooplankton through feeding, defecation, and excretion. Pomeroy (1984) states that there is more dissolved organic matter produced through excretion by zooplankton and lysis of phytoplankton than released by living phytoplankton in the oceans.

Algal-derived dissolved organic carbon is utilized rapidly by bacteria and converted into particulate material in the form of bacterial cells. So, once believed to serve only as mineralizers of organic compounds, bacteria are now recognized as having a great importance as producers of particulate organic carbon, and because they have access to a variety of dissolved and particulate substrates, bacteria can be significant producers of biomass (Pomeroy, 1984).

Bacteria and the Aquatic Food Web: Bacteria as Food

Bacteria constitute an important food source for protozoa. Protozoans may be the most important consumers of bacteria in nature and constitute an essential link in food chains between bacteria and higher trophic levels.

The rate of bacterial consumption by protozoa has been studied by several researchers. Barsdate et al. cited by Fenchel and Jorgensen (1974) found that during the initial exponential growth of ciliates they consumed 500-600 bacteria per individual per hour. When populations stabilized one ciliate consumed 100-200 bacteria per hour.

Bacteria cells as well as bacteria-detritus aggregates are consumed by some larger filter-feeding organisms: sponges, some polychaetes, cladocerans and diptera larvae (Fenchel and Jorgensen, 1974). Riemann and Bosselmann (1983) reported that Daphnia cucullata can filter 27% of the bacterial secondary production and Peterson and Hobbie (1978) found that Daphnia can feed efficiently on very small size natural bacteria populations. Borsheim and Olsen (1984) showed that when phytoplankton biomass is high, bacteria are a minor part on Daphnia pulex diet. As the algae biomass declines, bacterial biomass becomes the dominant food resource.

Carlholm (1984) suggests a feedback mechanism between aquatic microorganisms. The dissolved organic carbon released by the phytoplankton stimulates the bacterial population growth. Protozoa then feed on the bacteria and excrete ammonia which then is taken up by the phytoplankton.

Bacterial Productivity Determinations

Bacterial Activity Measurements

To determine the contribution of bacteria to the food chain and the importance of environmental effects on their activity, estimates of growth rates and biomass are essential. Due to the lack of suitable techniques, such measurements have not been possible in the past.

Lately, estimations of the bacterial production have been widely encouraged by the development of the ^{3}H-thymidine incorporation method of Fuhrman and Azam (1980). This method has been successfully used in both marine (Fuhrman, 1980; Fuhrman and Azam, 1980 and 1982; Hollibaugh, et al., 1980; Lancelot and Billen, 1984; Palumbo et al., 1984; Booth and Hoppe, 1985; Ducklow, 1983; Peele et al., 1985; Jeffrey and Paul, 1986) and freshwater ecosystems (Jordan and Likens, 1980; Ducklow et al., 1982; Kirchman, 1982; Riemann et al., 1982; Bell et al., 1983; Bell and Bern, 1983; Robarts and Sephton, 1983; Bell, 1984; Riemann, 1984; Riemann and Sondergaard, 1984; Lovell and Konopka, 1985; Toerien, 1985; Goulder, 1986; Scavia, 1986).

Tritiated Thymidine Technique

Fuhrman and Azam (1980) base their technique on measuring the incorporation of tritiated thymidine into the synthesis of new DNA. Thymidine is a specific precursor for DNA, and DNA is only synthesized by growing cells. The amount of thymidine incorporated is taken as a measure of growth (Wright, 1978; Karl, 1982; Fuhrman and Azam, 1982; Parsons, Maita and Lalli, 1984; Pollard and Moriarty, 1984). The technique is specific for the nonphotosynthetic bacteria and they found that virtually all of the actively growing bacteria take up thymidine. It is assumed that thymidine is rapidly and efficiently taken up by bacteria, is stable during uptake, is converted rapidly into nucleotide, and labels DNA with little or no dilution by intracellular pools (Kornberg, 1980). Because DNA is synthesized only in growing cells at a rate proportional to total biomass formation, the rate of DNA synthesis corresponds to the growth rate of the bacteria population (Van Es and Meyer-Reil, 1982).

The ^{3}H-thymidine method assumes that all microorganisms utilize exogenous thymidine in preference to de novo thymidine synthesis, an assumption confirmed by Fuhrman and Azam (1980 and 1982), Karl (1982), and Riemann (1984). Another assumption is that the ^{3}H-thymidine, in nanomolar concentrations, is assimilated exclusively by

bacteria, (Fuhrman and Azam, 1980 and 1982; Moriarty and Pollard, 1981; Bell et al., 1983; Pollard and Moriarty, 1984; Moriarty, 1986). A third assumption is that the naturally occurring thymidine does not affect the specific activity of the radiolabeled thymidine, an assumption that was questioned widely (Kornberg, 1980; Moriarty and Pollard, 1981; Karl, 1982; Riemann et al., 1982; Gude, 1984). Pollard and Moriarty (1984), adapted a technique known as isotope dilution to measure the changes of the specific radioactivity of the thymine precursor. The modification of Fuhrman and Azam (1980) technique by the isotope dilution approach (Pollard and Moriarty, 1984) gives a more reliable determination on the ^{3}H-thymidine incorporation.

Tritiated Thymidine and DNA Synthesis

The only function of thymine nucleotides in cells is their participation in DNA synthesis (Moriarty, 1986). There are two principal pathways (Fig. 1) for cell synthesis of thymine nucleotides. One is the de novo pathway in which the thymidine is synthesized in the form of dTMP (deoxythymidine monophosphate) by the enzyme thymidine synthetase from the precursor dUMP (deoxyuridine monophosphate). The other is the salvage pathway where free bases and nucleosides from DNA breakdown are converted (recycled) into thymine. Thymine, by the addition of

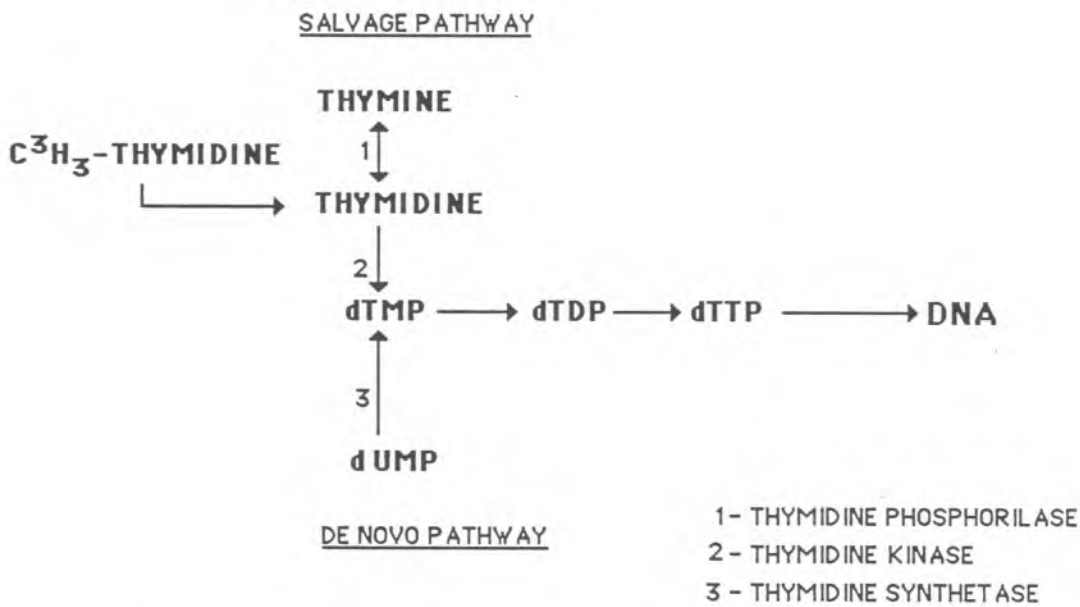

Fig. 1 Pathways of thymidine nucleotide metabolism. dTMP, dTDP, and dTTP, are thymidine mono-, di-, and triphosphate, respectively. dUMP is deoxyuridine mono phosphate. Modified from Moriarty (1986).

deoxyribose and the action of thymidine phosphorylase, can be transformed into thymidine, and this, into dTMP by the action of thymidine kinase (Kornberg, 1980; Moriarty, 1986). Regardless of the pathway, dTMP undergoes subsequent transformations into dTDP (deoxythymidine diphosphate), dTTP (deoxythymidine triphosphate), and DNA through the action of DNA polymerase.

Because the de novo biosynthetic pathway can contribute thymine to DNA it dilutes the radioactive thymine that is added from the ^{3}H-thymidine technique. This dilution results in underestimates when calculating cell synthesis.

Isotope Dilution Technique

Small pools of dTTP, though impossible to measure, constitute the immediate source of thymine for DNA synthesis, and, if present, they will dilute the labeled thymidine added thus lowering its specific activity. The isotope dilution technique allows one to measure the change in the specific radioactivity of the precursor (Pollard and Moriarty, 1984).

The principle of the technique is as follows: if there is no dilution by internal pools a number of counts should be incorporated into DNA from the added precursor of given specific activity and consequently is a measure of the rate

of DNA synthesis.

If the reciprocal of the observed counts is plotted against a known dilution (that is controlled by adding known labeled and non-labeled thymidine) a linear relationship is obtained with an intercept equal to the dilution given by the thymidine pool in the system (Pollard and Moriarty, 1984). It is not strictly a pool of thymidine, but represents the sum of all pools that dilute the tritiated thymidine prior to incorporation into DNA (Moriarty, 1986).

An alternate approach to solve this problem rests on the fact that bacteria can regulate the synthesis of thymine precursors. If the labeled thymidine is supplied at sufficiently high concentrations, de novo synthesis is inhibited (Riemann et al., 1982; Pollard and Moriarty, 1986; Moriarty, 1986). Several concentrations have been recommended, 5 nM in lake water (Bell et al., 1983), 5-10 nM in seawater (Fuhrman and Azam, 1982; Kirchman et al., 1982) and 20 nM (Moriarty, 1986).

Bacterial Biomass

The bacterial biomass (i.e., the number or mass of potential producers) is an important variable when studying bacterial production. To estimate bacterial biomass, direct counts of the cells and size estimates are necessary. The

development of the Acridine Orange Direct Counts (AODC) technique by Hobbie et al. (1977) using a fluorescent dye and epifluorescent microscope has permitted reliable measurements. This technique is the basic method of Francisco et al. (1973) as modified by Jones (1974).

After staining, forms with clear outline and recognizable bacterial shape and bright orange or green fluorescence are counted as bacterial cells, reducing in this way subjectivity errors (Van Es and Meyer-Reil, 1982). The epifluorescence technique allows the study of the different types of bacteria present in a natural sample (cocci, bacilli, streptobacilli) and moreover, the ratio of free-floating to particle-attached bacteria can be determined relatively easily (Van Es and Meyer-Reil, 1982). This method is useful when counting very small cocci, 0.2 to 0.8 um in diameter, that can not be seen when using nonfluorescence techniques (Ralph and Hobbie, 1975, cited by Van Es and Meyer-Reil, 1982)

Bowden (1977) found that bacterial counts done using the scanning electron microscope (SEM) agree closely with the fluorescing bacteria counted using the AODC technique. He suggests the use of the latter for routine ecological sampling because of considerable savings in time.

Also microscopic determination of cell dimensions have been verified with SEM (Van Es and Meyer-Riel, 1982). However, Fuhrman (1981) concluded that epifluorescence microscopy is more accurate than SEM for these measurements. During preparation for SEM, cells shrink a variable amount.

METHODS

The variables determined in each sample were water temperature, turbidity, chlorophylls *a*, *b*, and *c*, dissolved and total organic carbon, bacterial growth rates, cell concentration, and cell volume.

Sampling

Water samples were collected from the middle of Lake Brazos near the Baylor University Marina. Lake Brazos is a "town lake" located in McLennan County, Texas. It divides the city of Waco into two areas. The lake was created by the impoundment of the Brazos River with a small dam. The lake is 15.17 km long with an average width of 94.8 m, and a maximum depth of 8 m. The Brazos River brings water from a series of salt springs in west Texas making the water of the lake unusual for waters of this region (Table 1).

Sampling began on April 22, 1986 and terminated on June 27, 1986. Sampling dates were selected to include a variety of water turbidities. Nine sampling series were carried out between these dates. For each series an integrated sample was taken from the surface 0.5 m with a plastic tube (5 cm, inside diameter), placed in a sterile polyethylene bottle at

Table 1. Average chemical characteristics of Lake Brazos. (Data are from Texas Water Quality Board)

VARIABLE	1982	1983	1984
pH	8.0	7.9	7.3
Alkalinity (mg CaCO l^{-1})	169.0	160.0	140.0
Nitrogen, Ammonia Total (mg l^{-1})	0.01	0.05	0.04
Nitrogen Nitrate Total (mg l^{-1})	0.01	0.33	0.18
Phosphate as Ortho (mg l^{-1})	0.16	0.48	0.24
Phosphorus, Total (mg l^{-1})	0.30	0.22	0.12
Total Organic Carbon (mg l^{-1})	12.3	17.0	18.0
Chloride (mg l^{-1})	126.0	85.0	113.0

10:00 h, and transported to the laboratory. Care was taken to protect the sample from direct exposure to the sunlight. Processing was begun within 30 minutes of collection.

At the laboratory, sub-samples for bacterial counts were removed immediately after shaking the sample to insure a homogeneous bacterial suspension. These were preserved with an equal volume of 4% formalin (final concentration 2%) and kept cold in the dark until acridine orange direct counts (AODC) were made.

Turbidity Measurement

Turbidity was measured in nephelometer turbidity units (NTU) with a nephelometer (Hach, model 2100A) using formazin standards.

Chlorophyll Measurement

Relative phytoplankton biomass was estimated as chlorophyll *a*. Chlorophyll (*a*, *b*, and *c*) concentrations were determined from one liter samples using a narrow band pass spectrophotometer (Beckman, model 25) according to the technique described by Lind (1979) and the trichromatic equations of Strickland and Parsons (1968).

Dissolved and Total Organic Carbon Measurements

Sample preparation for dissolved organic carbon (DOC), and total organic carbon (TOC) was done according to the Menzel and Vaccaro (1964) technique. Inorganic carbon was removed from the sample by acidifying with 3% phosphoric acid and then sparging with N_2. The remaining organic carbon was treated with a strong oxidant (potassium persulfate) at elevated temperature and pressure in sealed ampoules. The amount of CO_2 generated from the organic carbon was measured with an infrared analyzer (Beckman, model 215A) using CO_2 standards. For DOC determination, the sample was filtered through a prewashed 0.2 um pore size membrane filter. Five replicates per sample and per variable (DOC, TOC) were used to determine the organic carbon content.

Bacterial Growth Rates, Tritiated Thymidine Method

Thymidine incorporation measurements were done in the laboratory. The treatment of the samples was done in autoclaved screw-top test-tubes. A sterilized volume-adjustable dispenser was used to fill rapidly the test tubes with the water sample.

Five replicates of each sample treatment were incubated with 18 nM ^{3}H-methyl-thymidine (55 Ci per mmol, ICN Radiochemicals) concentration. Five replicates gave a coefficient of variation range between 21 and 6% in samples with live bacteria and a range between 82 and 18% in the control samples with killed bacteria. Killed controls were prepared with formalin-azide solution, 5% final concentration.

The five isotope dilution treatments were:

TUBE 1- 10 ml killed sample + 10 ul labeled thymidine

TUBE 2- 10 ml sample + 10 ul labeled thymidine

TUBE 3- 10 ml sample + 10 ul labeled thymidine + 10 ul unlabeled thymidine

TUBE 4- 10 ml sample + 10 ul labeled thymidine + 20 ul unlabeled thymidine

TUBE 5- 10 ml sample + 10 ul labeled thymidine + 30 ul unlabeled thymidine

Each treatment was replicated five times.

The test tubes were placed in a dark water bath at constant temperature (25-26 C) for 30 minutes. The incubation and bacterial growth was terminated by adding formalin-azide solution (5% final concentration) to each tube, then the tubes were immediately placed in crushed ice.

Each sample was filtered through an ice cold 25 mm diameter nitrocellulose filter (.45 um pore size, Schleicher and Schuell). The collected material on the filter was washed with 30 ml of ice cold 5% trichloroacetic acid under continuous gentle (less than 8 in Hg) filtration pressure (Riemann, 1984) to remove the unused labeled thymidine by the bacteria. The filters were then placed in scintillation vials containing ethyl acetate (1 ml) to dissolve the filter. Ten ml of scintillation fluid (Beckman, HP/b) were then added and the radioactivity counted in a liquid scintillation counter (Beckman, model LS 1800). Samples were counted to a 1% confidence level (2 sigma = 1.00) with two cycles repetition for each series. The second cycle data were used to eliminate possible spurious autofluorescence.

To determine the sample activity, or disintegrations per minute (DPM), the H-number of the sample was calculated and external quench correction applied. The H-Number is the difference between the pulse heights of the sample relative to an unquenched standard. Quench is the reduction of energy transfer efficiency in a sample. A quench curve was made by filtering progressively greater volumes of lake water through membrane filters. These filters are placed in scintillation vials with a known concentration of isotope and DPM activity is determined. The quench curve relates the extent of quenching in any sample, as shown through its

H-Number to a counting efficiency (Beckman, 1982).

Calculation of Bacterial Growth Rates

The reciprocal of ^{3}H-thymidine incorporated into DNA in DPM is plotted against the total thymidine concentration used during the incubation (labelled and unlabeled). A linear relationship is obtained with a negative intercept that represents the dilution of the added thymidine by the thymidine pool in the system. If there is no dilution by any sources (i.e., de novo synthesis) other than the unlabeled thymidine added, the plot will pass through zero (Pollard and Moriarty, 1984).

The real specific activity of the labeled thymidine used during the incubation, including possible isotope dilution is calculated using this plot, according to Pollard and Moriarty (1984):

SA = uCi/conc. added + line intercept conc.

Where: uCi is the added ^{3}H-thymidine initial activity; conc. added is the total thymidine concentration added to the sample in nmol; and line intercept conc. is obtained from the negative intercept of the plotted line in nmol.

Bacterial production of cells per liter per hour is estimated according to Pollard and Moriarty (1984) as follows:

$$\text{Cell production } l^{-1}\ h^{-1} =$$

$$((dpm/2.22X10^{6}/sa)(1/time)(vol/1000)(1X10^{-9})\ (2.1X10^{18})$$

Where:

dpm is obtained from the sample with only ^{3}H-thymidine added,

$2.22X10^{6}$ is the number of DPM uCi^{-1}

sa is the specific activity of the isotope as determined above,

time is the incubation time in hours,

vol is the sample volume used in ml,

$1X10^{-9}$ is a conversion factor, nmoles to moles,

$2.1X10^{18}$ is the relation of cells produced per mole thymidine incorporated.

Bacterial Cell Concentration and Bacterial Volume Measurement

Bacterial counts and size measurements were made by using the acridine orange direct count (AODC) method of Hobbie et al. (1977) with a minor change. A drop of water was used between the filter and the cover slip. This gave better contrast between the bacteria cell and the background than the immersion oil.

The acridine orange solution 0.1% (Sigma Chemicals Co.) as well as any water used for dilution of the sample or for rinsing filters was filtered through 0.2 um Nucleopore filters each day to avoid contamination of the sample with external bacteria.

Nucleopore filters (0.2 um pore size, 25 mm diameter) were stained by soaking for 24 hours in a solution of 2 g of Irgalan Black (Ciba-Geigy) in 1 l of 2% acetic acid and rinsed in sterilized water immediately before use.

The stained filter was placed on top of a cellulose filter to give a better vacuum distribution (less than 8 in Hg). One milliliter of sample was brought to 2 ml in the filtration unit with sterile water to ensure an even cell distribution. The sample was filtered after incubating with one drop of AO 0.1% for 2 minutes. Damp filters were placed on glass slides, covered with one drop of water, a cover slip, and one drop of low fluorescence immersion oil.

Free living bacteria and bacteria attached to particles were counted separately and the combination of each constitutes the total bacterial concentration of the sample. The number of bacteria per milliliter was estimated from a count of 20 fields in duplicate per each sample with a minimum of 20 cells per field. Counts were made at 1500x magnification. Counts of attached bacteria were corrected for hidden bacteria by multiplying the count by two,

considering that an equal number on top of the particle could be found below it (Clarke and Joint, 1986; Chrzanowski, personal communication).

Size was measured on free cocci, free bacilli, attached cocci, and attached bacilli. The length and width of the rods and the diameter of the cocci were measured to estimate the volume.

Biomass Estimates

The bacterial biomass was calculated as the sum of the biomass of the free living bacteria and the attached bacteria. The biomass of each was obtained by multiplying the average volume by the average number of cells and transforming to carbon units, using the relation of $1.21X10^{-13}$ g C um^{-3} (Fuhrman and Azam, 1980; Riemann et al., 1982).

Data Analyses

In this study, multiple regression analysis was used to determine the relation of the environmental variables measured to the bacterioplankton productivity. Regression analysis measures the relationship between a dependent variable (bacterioplankton) and one or more independent variables (environmental). Statistical data analyses were

done with the statistical programs of SPSSx (SPSS, Inc. 1983), using the regression stepwise procedure. The regression first enters the variables into the equation. Then the specified variables are tested upon removal as specified by the stepwise selection. Because all the independent variables included in a multiple regression equation do not necessarily have a significant effect on the dependent variable, in the stepwise method each variable is tested in order to enter the regression equation (tolerance criterion). The probability of F-to enter (PIN) a variable from the equation used in the study was 0.05.

RESULTS

Turbidity had the highest correlation with bacterial cell synthesis. Multiple regression analysis showed that 63.6% of the changes in the bacterial cell synthesis were explained by the water's turbidity, 82.3% of the changes were explained when combining turbidity with the attached cell biomass and 95.6% were explained when combining turbidity, attached cell biomass and total organic carbon.

Changes in specific production, that is production per biomass, were explained primarily by the turbidity (r^2=.76), and the combination of turbidity and DOC explained 90.3%.

The absolute bacterial productivity changes were not significantly explained by any of the environmental variables measured during the study.

Environmental Variables

Because of local heavy thunderstorms and flooding before and during some of the sampling dates the turbidity fluctuated greatly (between 6.4 and 55 NTU, Table 2). Also, the total organic carbon (TOC) ranged between 8.4 and 13.2

TABLE 2. Turbidity and temperature measurements for Lake Brazos.

SERIES	TURBIDITY ntu	TEMPERATURE C
I	25.0	22.0
II	14.0	25.0
III	30.0	25.0
IV	19.0	25.2
V	53.0	27.0
VI	55.0	26.5
VII	28.0	27.0
VIII	6.4	27.5
IX	11.0	27.2

mg C l^{-1} and the dissolved carbon (DOC) concentrations ranged between 8.0 to 11.0 mg C l^{-1} (Table 3). Phytoplankton biomass, as estimated by chlorophyll *a* concentration, varied from a 2.5 to 6.9 ug Chl l^{-1} (Table 4). Water temperature increased gradually during the study period (May-June 1986), and ranged between 22 to 27.5 C (Table 2).

Turbidity and pigment concentration showed a reciprocal relationship. Probably, nutrients washed off by the rain stimulated algal growth as soon as the light penetration increased.

As expected, the higher concentrations of TOC corresponded with the turbidity peaks and were negatively correlated with temperature (Table 5). There was a strong positive correlation between DOC and chlorophyll *c* (indicative of diatoms), (Table 5). There was no correlation between turbidity and DOC, suggesting that the dissolved organic carbon in the water was due mainly to photosynthetic activities, algae lysis, or excretion.

The Bacterial Population

Coccoid bacteria were the predominant type present. They had an average cell volume of 0.063 um^3 when free and 0.075 um^3 when attached. The bacilli, though less

Table 3. Organic carbon measurements for Lake Brazos, in mg l^{-1}.

SERIES	TOC	DOC
I	13.2	8.6
II	9.0	7.0
III	11.0	10.0
IV	11.0	11.0
V	11.8	9.2
VI	11.0	7.8
VII	10.0	9.8
VIII	8.4	8.0
IX	8.4	8.4

Table 4. Chlorophyll a and c measurements for Lake Brazos in ug l^{-1}.

SERIES	CHL a	CHL c
I	2.52	ND
II	5.12	ND
III	2.80	0.45
IV	4.15	0.50
V	3.80	ND
VI	5.50	ND
VII	6.9	1.18
VIII	4.4	ND
IX	3.9	ND

ND = Not detected

Table 5. Correlation coefficients (r) shown by the environmental variables measured for Lake Brazos (significance of r when $p<0.05 = 0.66$).

	DOC	TOC	CHLc	CHLa	TURB
TEMP	0.04	-0.66	0.13	0.55	0.06
TURB	-0.09	0.61	-0.02	0.09	
CHLa	0.45	-0.43	0.53		
CHLc	0.92	0.00			
TOC	0.06				

numerous were larger and had an average volume of 0.22 um^3 when free and 0.17 um^3 when attached. Small curved (lunate) bacteria were found occasionally. Filamentous bacteria (streptobacilli) were observed in very small numbers. The average bacterial volume including all three forms ranged between 0.0854 and 0.2252 um^3 (Table 6).

Bacterial cell numbers remained fairly constant among the sampling series. They ranged from $1.67X10^9$ to $3.45X10^9$ cells l^{-1}. Most of the bacteria were free living, constituting an average of 84.6% of the total population (Table 6).

The free bacterial biomass ranged between 12.47 to 68.78 ug C l^{-1} and the attached cells biomass ranged between 1.98 to 10.59 ug C l^{-1} (Table 7).

The free bacterial abundance was correlated negatively with the water's turbidity. The numbers of attached bacteria were highly correlated with the TOC and correlated negatively with the temperature. The total volume of the free cells was highly related to the chlorophyll c concentration (Table 8). Multiple regression analysis explained 52.3% of the free cells volume changes by the chlorophyll c concentration.

Table 6. Abundance (10^9 cells l^{-1}) and average cell volume (um^3) from bacterial populations in Lake Brazos (May-June).

	ABUNDANCE			VOLUME		
SERIES	FREE	ATTACHED	TOTAL	FREE	ATTACHED	TOTAL
I	1.58	.561	2.14	0.124	0.153	0.139
II	1.56	.262	1.81	0.191	0.124	0.158
III	1.37	.304	1.67	0.147	0.096	0.122
IV	2.56	.417	2.97	0.123	0.069	0.096
V	1.39	.365	1.76	0.074	0.099	0.085
VI	1.50	.279	1.78	0.076	0.095	0.086
VII	1.78	.294	2.07	0.283	0.105	0.194
VIII	3.27	.181	3.45	0.174	0.276	0.225
IX	1.86	.202	2.07	0.117	0.079	0.098

Table 7. Bacterial biomass (ug C l^{-1}) in Lake Brazos, during May-June, 1986.

SERIES	FREE	ATTACHED	TOTAL
I	23.70	10.59	34.29
II	36.06	3.84	39.90
III	24.39	3.60	27.98
IV	38.10	3.56	41.66
V	12.47	4.45	16.92
VI	13.90	3.26	17.16
VII	60.84	3.81	64.85
VIII	68.78	6.15	74.93
IX	26.40	1.98	28.38

Table 8. Correlation coefficients (r) shown by the various environmental variables measured for Lake Brazos (significance of r when $p<0.05 = 0.66$).

	DOC	TOC	CHLC	CHLA	TURB	TEMP
FCONC	0.10	-0.47	0.01	0.09	-0.61	0.31
ACONC	0.18	0.91	0.02	-0.46	0.29	-0.78
FVOL	0.59	-0.40	0.72	0.57	-0.46	0.11
AVOL	-0.37	-0.28	-0.27	-0.06	-0.41	0.08
FBIOMASS	-0.28	0.47	-0.21	0.02	-0.45	0.36
ABIOMASS	-0.22	0.56	-0.22	-0.44	-0.08	-0.68
CSYN	-0.31	0.56	-0.20	0.10	0.80	-0.02
PB	-0.44	0.55	-0.36	-0.03	0.88	-0.02
PROD	0.00	-0.03	0.18	0.38	-0.08	0.00

FCONC = free cells concentration
ACONC = attached cells concentration
FVOL = free cells volume
AVOL = attached cells volume
FBIOMASS = free cells biomass
ABIOMASS = attached cells biomass
CSYN = cell synthesis
PB = specific production
PROD = bacterial secondary productivity

Bacterial Cell Synthesis and Productivity

The rate of bacterial cell synthesis ranged from $1.97X10^{7}$ to $4.79X10^{7}$ cell l^{-1} h^{-1}, (Table 9). The ranges of specific production were 0.006 to 0.03 ug C ug C^{-1} l^{-1} h^{-1}, (Table 10). The bacterial population turnover time varied between 1.4 to 7.4 days (Table 10). The bacterial productivity ranged between 0.23 to 0.83 ug C l^{-1} h^{-1}, (Table 10).

Table 9. Bacterial cell synthesis (cell l^{-1} h^{-1}) determined by the tritiated thymidine incorporation method.

SERIES	CELL SYNTHESIS
I	$4.14X10^7$
II	$2.60X10^7$
III	$2.43X10^7$
IV	$1.97X10^7$
V	$4.79X10^7$
VI	$4.68X10^7$
VII	$3.46X10^7$
VIII	$2.92X10^7$
IX	$2.25X10^7$

Table 10. Production (ug C l^{-1} h^{-1}), specific production (ug C ug C^{-1} l^{-1}) and doubling time (days) estimates for Lake Brazos bacteria.

SERIES	PRODUCTION	SPECIFIC PRODUCTION	DOUBLING TIME
I	.7073	.02063	2.02
II	.5046	.01265	3.29
III	.3643	.01302	3.20
IV	.2333	.00560	7.44
V	.5093	.03010	1.38
VI	.4938	.02878	1.45
VII	.8265	.01279	3.26
VIII	.8088	.01079	3.86
IX	.2717	.00957	4.35

DISCUSSION

Environmental variables will affect bacterial cell synthesis and specific production. This relationship will determine bacterial productivity in freshwater. The main point of my study was to measure the relative effects of those variables on the bacterial productivity in Lake Brazos.

Water turbidity was the most important variable affecting bacterial production in this study. My results showed that water turbidity explained 63.6% of the variation on the bacterial cell synthesis, and 76% of the variation on the bacterial specific production. Others have suggested the importance of suspended particles to bacterial populations (Pearl and Goldman, 1972; and Paerl, 1975; cited by Toerien, 1985). Toerien (1985) measured bacterial activity in Wuras Dam, a highly turbid South African reservoir, using the glucose assimilation technique. He did not find any significant relationship between turbidity and bacterial population.

Heterotrophic bacterial growth ultimately depends on the supply of organic nutrients from autochthonous and or allochthonous sources (Gude, Haibel, Muller, 1985).

Bacterial cell synthesis (cell l^{-1} h^{-1}) increased shortly after DOC increases, and the peak rates of cell synthesis coincided with the minimum concentration of DOC. Apparently the dissolved organic nutrients from both allochthonous and autochthonous sources stimulated the bacterial synthesis that then diminished the DOC in the water (Fig. 2). Fuhrman and Azam (1980) found that the bacteria can grow fast enough in coastal waters to utilize a significant fraction of the dissolved organic carbon.

Organic products of the diatom population rather than allochthonous sources apparently were the main source of DOC in Lake Brazos. A high correlation between chlorophyll c and DOC (r=0.92) was found. The phytoplankton density peaks occurred when turbidity was low (Fig. 3). Similarly others have shown that phytoplankton photosynthetic activity is an important source of organic nutrients for the bacterial populations in both freshwater and marine ecosystems (Fuhrman et al., 1980; Blaauboer et al., 1982; Cole, 1982; Coveney, 1982; Riemann et al., 1982; Bell et al., 1983; Bird and Kalff, 1984; Brock and Clyne, 1984; Lovell and Konopka, 1985; Peele et al., 1985; Goulder, 1986).

The cell synthesis showed a reciprocal relation with the bacterial biomass (Fig. 4). Borsheim and Olsen (1984), showed that when the algal biomass is low, the bacterial biomass constitutes the dominant food resource for

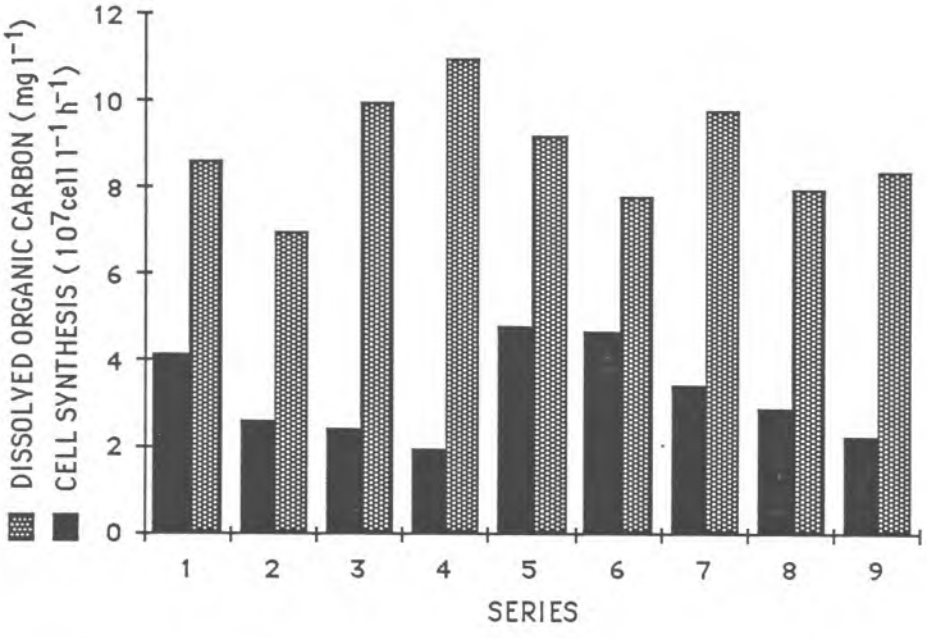

Fig. 2 Relation between DOC measurements and bacterial cell synthesis in Lake Brazos. High DOC concentrations coincide with low rates on cell synthesis.

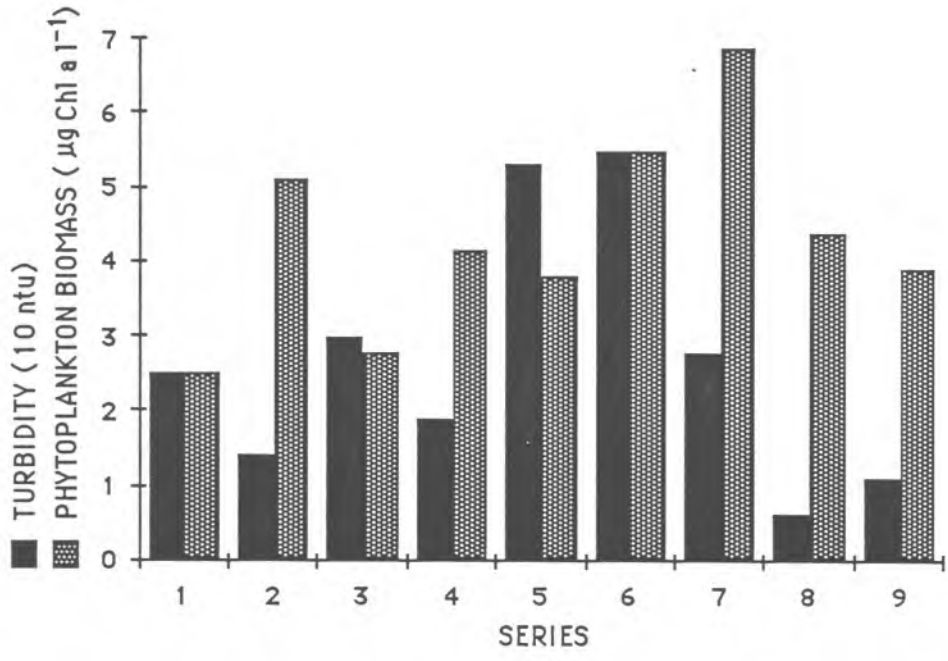

Fig. 3 Relationship shown by the water turbidity and the phytoplankton density in Lake Brazos. Peak phytoplankton abundance occurred during low turbidity periods.

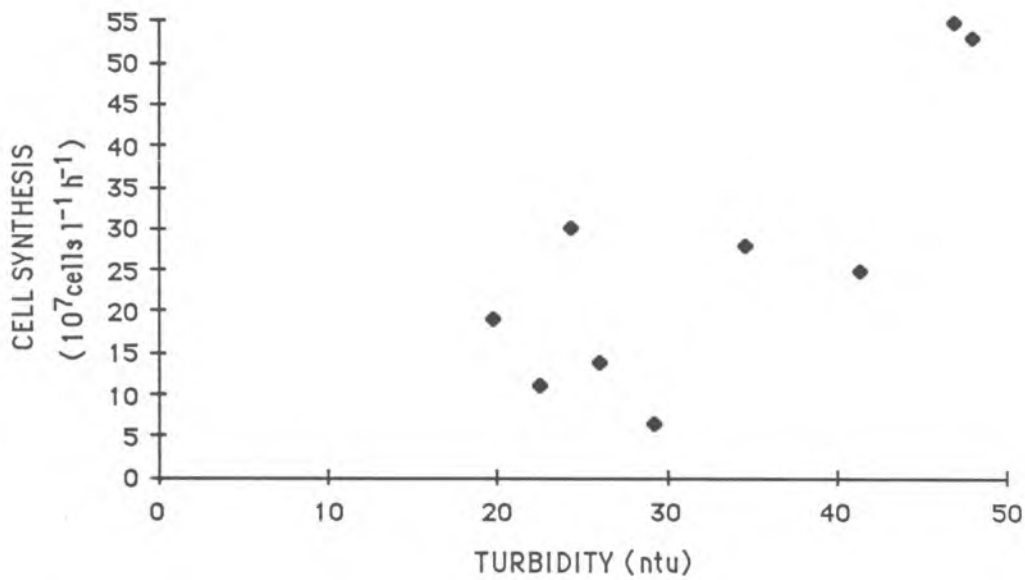

Fig. 4 Scattegram showing the relationship between bacterial cell synthesis and turbidity in Lake Brazos.

zooplankton. During turbidity peaks low phytoplankton biomass was present and this coincided with high rates on cell synthesis and low bacterial biomass (Fig. 5). Pedros-Alio and Brock (1983) calculated that zooplankton feeding can consume 1-60% of the bacterial biomass daily. It also has been shown that flagellates consume a considerable portion of the bacterial productivity in some freshwater ecosystems (Pomeroy, 1984; Gude et al., 1985). Another consideration is the sinking rate. Sinking is negligible for free living bacteria but can be much greater for attached bacteria (Lovell and Konopka, 1985). Ducklow et al. (1982) have measured settling rates of 0.1 to 1.0 m per day for attached bacteria in the Hudson River plume. Also, Palumbo et al. (1984) suggested that attached bacteria are more available to predators. A combination of these factors may have accounted for the low biomass during high cell synthesis periods.

The range of bacterial production obtained in this study varied between 0.23 ug C^{-1} h^{-1} and 0.83 ug C l^{-1} h^{-1}. A similar range of production values were obtained for some other lakes (Table 11).

The bacterial population doubling time was between 1.4 to 7.4 days during May and June. Riemann et al. (1982) reported a range between 7 and 37 days, and Jordan and Likens (1980) reported an annual doubling time range in

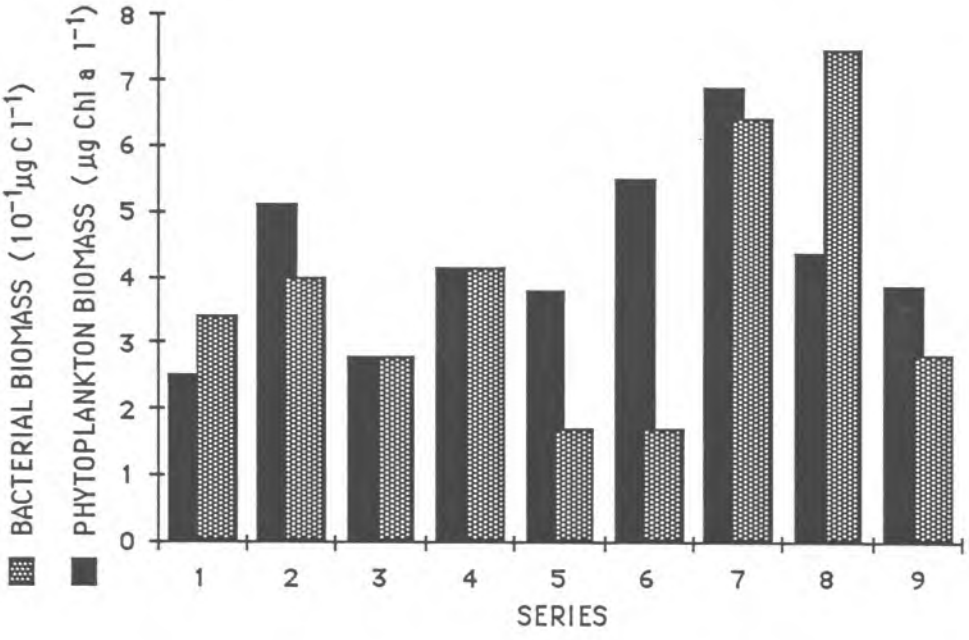

Fig. 5 Relationship found between bacterial biomass and phytoplankton biomass in Lake Brazos. In most cases bacterial increases in biomass coincide with phytoplankton biomass increases.

Table 11. Bacterial production (ug C l^{-1} h^{-1}) estimates in aquatic ecosystems.

LOCATION	PRODUCTION	SPECIFIC GROWTH RATE (day^{-1})	REFERENCE
SEAWATER			
North Sea	0.80 -0.24		1
Antarctic	0.002-0.121	0.007-0.46	2
New York Bight	0.294-0.556	0.09	3
California Coast	0.196-2.21	0.050-2.3	4
Chesapeake Bay	0.292-3.13	1.10 -6.9	5
Moreton Bay, East Australia	0.30	1.3	6
Great Barrier Reef	0.375-1.83	0.050-6.9	7
FRESHWATER			
Norrviken	0.20 -7.1		8
Mendota	0.30 -8.3		9
Lake Almind	0.325		10
Lake Orn	1.35		10
Lake Esrom	0.283		10
Lake Mosso	0.454		10
Lake Hodges	0.071		10
Crooked Lake	0.107		11
Little Crooked	0.176		11
Lake Brazos	0.23 -0.83	0.130-0.72	12

REFERENCE

1 Lancelot and Billen, 1984
2 Fuhrman and Azam, 1980
3 Ducklow et al., 1982
4 Fuhrman and Azam, 1982
5 Ducklow, 1982
6 Moriarty and Pollard, 1982
7 Moriarty et al., 1982
8 Bell et al., 1982
9 Pedros-Alio and Brock, 1982
10 Riemann and Sondergaard, 1984
11 Lovell and Konopka, 1985
12 This study

Mirror Lake from 1.2 to more than 100 days. The variation in specific production in Lake Brazos was explained 76.7% by the turbidity (Fig. 6) and 90.3% when combining turbidity and DOC. This organic enrichment effect on turnover rates also has been reported by Wright (1978).

The small percent of attached cells found (15.4±5.7) is not unusual (Fig. 7). Hobbie and Wright (1979) indicate that most planktonic bacteria will be unattached. Pedros-Alio and Brock (1983) found between 1- 30% attached to particles in Lake Mendota. Simon and Tilzer (1982) report 5% in Lake Constance. Laanbroek and Verplanke (1986) found that the attachment of bacteria follows a seasonal pattern. They found low percentages of attachment during spring (April-July). Lake Brazos sampling was during the months of May and June. Attachment of bacteria to particles is often viewed as an advantageous starvation response (Jeffrey and Paul, 1986). In Lake Brazos, DOC was available and explained, in combination with turbidity, 90.3% of the variation in the bacterial population doubling time.

The sizes of bacterial cells observed in Lake Brazos are similar to those found in other lakes (Hobbie and Wright, 1979; Pedros-Alio and Brock, 1983). The high correlation between the chlorophyll *c* concentration and the volume of the free living cells (r=0.72), suggests that the activity of diatoms provided nutrients that stimulated the

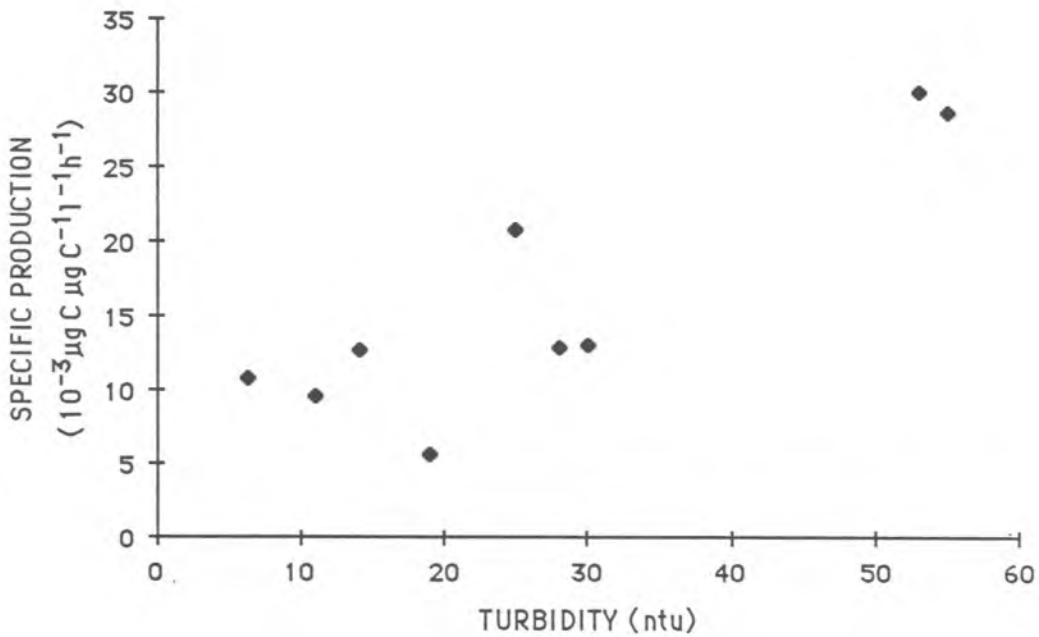

Fig. 6 Relationship shown between turbidity and specific production in Lake Brazos.

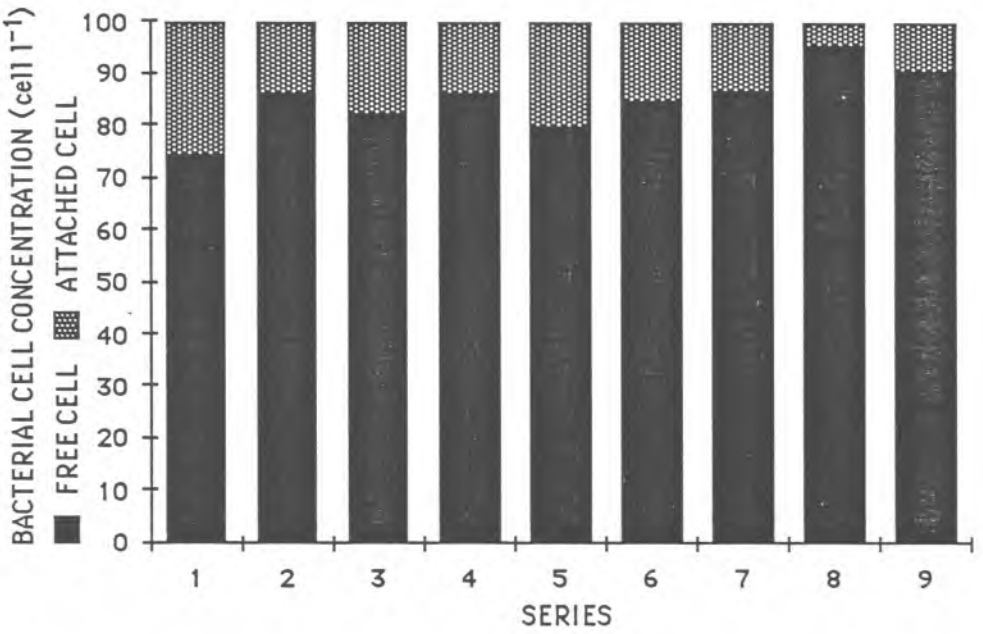

Fig. 7 Ratio of free living bacteria to particle attached bacteria in Lake Brazos during May and June 1986.

cell size growth of the free bacteria.

In the present study, the bacterial cell synthesis and the specific production of the bacterial population appear to be controlled by the turbidity and dissolved organic carbon present in the water. This is especially significant to our understanding of reservoir ecosystems which are characterized by high turbidity and dissolved organic carbon concentrations. The high productivity of the higher trophic levels usually found in such turbid waters with reduced photosynthesis may depend on the bacterial production.

LITERATURE CITED

Alexander, M. 1977. Advances in Microbial Ecology. Vol. 1. Plenum Press, New York. 268 pp.

Beckman. 1982. LS 5800 series liquid scintillation systems: preliminary manual. Beckman Instruments, Inc., Fullerton, Ca.

Bell, R. T. 1984. Thymidine incorporation rates and bacterioplankton dynamics during early spring in Lake Erken. Arch. Hydrobiol. Beih. 19:81-89.

Bell, R. T., G. M. Ahlgren, and I. Ahlgren. 1983. Estimating bacterioplankton production by measuring ^{3}H-thymidine incorporation in a eutrophic Swedish lake. Appl. Environ. Microbiol. 43:200-209.

Bell, R. T., and L. Bern. 1983. Bacterioplankton dynamics in eutrophic lake Norrviken (Sweden). Verh. Int. Ver. Limnol. 22:735-741.

Bell, W., and R. Mitchell. 1972. Chemotactic and growth responses of marine bacteria to algal extracellular products. The Biological Bulletin--Marine Biological Laboratory. 143:265-277.

Bell, W. H., and E. Sakshaug. 1980. Bacterial utilization of algal extracellular products. A kinetic study of natural populations. Limnol. Oceanogr. 25: 1021-1034.

Bird, D. F., and J. Kalff. 1984. Empirical relationships between bacterial abundance and chlorophyll concentration in fresh and marine waters. Can. J. Fish. Aquat. Sci. 41:1015-1021.

Blaauboer, M. C. I., and R. Van Keulen, T. H. E. Cappenberg. 1982. Extracellular release of photosynthetic products by freshwater phytoplankton populations with special reference to the algal species involved. Freshwater Biology. 12:559-572.

Booth, W. E., and H. G. Hoppe. 1985. Epiphytic bacterial activity on different macroalgal species determined by tritiated thymidine incorporation. Botanica Marina 27:47-56.

Borsheim, K. Y., and Y. Olsen. 1984. Grazing activities by Daphnia pulex on natural populations of bacteria and algae. Verh. Internat. Verein. Limnol. 22:584-590.

Bowden, W. B. 1977. Comparison of two direct-count techniques for enumerating Aquatic Bacteria. Appl. Environ. Microbiol. 3:1229-1232.

Brock, T., and J. Clyne. 1984. Significance of algal excretory products for growth of epilimnetic bacteria. Appl. Environ. Microbiol. 47:731-734.

Bull, A. T., and J. H. Slater. 1982. Microbial Interactions and Communities. Vol. 1. Academic Press, London. 567 pp.

Campbell, R. 1983. Microbial ecology. Blackwell Scientific Publications, Oxford. 191 pp.

Carlholm, M. 1984. Heterotrophic, free-living protozoa: neglected microorganisms with an important task in regulating bacterial populations. Pages 321-326. In: Klug and Reddy (eds), Current Perspectives on Microbial Ecology. Am. Soc. for Microbiology, Washington, D. C. 709 pp.

Clarke, K. R., and J. R. Joint. 1986. Methodology for estimating numbers of free-living and attached bacteria in estuarine water. Appl. Environ. Microbiol. 51:1110-1120.

Cole, J. J., G. E. Likens, and D. L. Strayer. 1982. Photosynthetically produced dissolved organic carbon: an important carbon source for planktonic bacteria. Limnol. Oceanogr. 27:1080-1091.

Coveney, M. F. 1982. Bacterial uptake of photosynthetic carbon from freshwater phytoplankton. Oikos. 38:8-20.

Currie, D. J., and J. Kalff. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorous. Limnol. Oceanogr. 29:298-310.

Ducklow, H. W., 1983. Production and fate of bacterial in the oceans. BioScience. 33:498-501.

Ducklow, H. W., D. L. Kirchman, and G. T. Rowe. 1982. Production and vertical flux of attached bacteria in the Hudson River plume of the New York Bight as studied with floating sediment traps. Appl. Environ. Microbiol. 43: 769-776.

Fenchel, T. M. 1986. The ecology of heterotrophic microflagellates. Pages 59-98. In: Marshal, K. C. (ed), Advances in Microbial Ecology. Vol. 9. Plenum Press, New York. 402 pp.

Fenchel, T. M., and B. B. Jorgensen. 1977. Detritus food chains of aquatic ecosystems: the role of bacteria. Pages 1-49. In: Alexander, M. (ed), Advances in Microbial Ecology. Vol. 1. Plenum Press, New York. 268 pp.

Fogg, G. E. 1971. Extracellular products of algae in freshwater. Ergebn. Limnol. 5:1-25.

Forsberg, C., and S. O. Ryding. 1980. Eutrophication parameters and trophic state indices in 30 Swedish waste-receiving lakes. Arch. Hydrobiol. 89:189-207.

Fuhrman, J. A. 1981. Influence of method on the apparent size distribution of bacterioplankton cells: epifluorescence microscopy compared to scanning electron microscopy. Mar. Ecol. Progr. Ser. 5:103-106.

Fuhrman, J. A., J. W. Ammerman, and F. Azam. 1980. Bacterioplankton in the coastal eutrophic zone: distribution, activity and possible relationships with phytoplankton. Mar. Biol. 60:201-207.

Fuhrman, J. A., and F. Azam. 1980. Bacterioplankton secondary production estimates for coastal waters of British Columbia, Antarctica, and California. Appl. Environ. Microbiol. 39:1085-1095.

Fuhrman, J. A., and F. Azam. 1982. Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. Mar. Biol. 66:109-120.

Francisco, D. E., R. A. Mah, and A. C. Rabin. 1973. Acridine Orange-Epifluorescent technique for counting bacteria in natural waters. Trans. Am. Microsc. Soc. 92:416-421.

Goulder, R. 1986. Seasonal variation in the abundance and heterotrophic activity of suspended bacteria in two lowland rivers. Freshwater Biology. 16:21-37.

Grant, W. D., and P. E. Long. 1981. Environmental Microbiology. John Wiley and Sons, New York. 215 pp.

Gude, H. 1984. Test for validity of different radioisotope activity measurements by microbial pure and mixed cultures. Arch. Hydrobiol. Beih. Ergebn. Limnol. 19:257-266.

Gude, H., B. Haibel, and H. Muller. 1985. Development of planktonic bacterial populations in a water column of Lake Constance (Bodensee- Obersee). Arch. Hydrobiol. 105:59-77.

Hobbie, J. E., R. J. Daley, and S. Jasper. 1977. Use of nucleopore filters for Ccounting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. 33:1225-1228.

Hollibaugh, J. T., J. A. Fuhrman, and F. Azam. 1980. Radioactively labeling of natural assemblages of bacterioplankton for use in trophic studies. Limnol. Oceanogr. 25:172-181.

Jeffrey, W. H., and J. H. Paul. 1986. Activity of an attached and free-living Vibrio sp as measured by thymidine incorporation, P- Iodonitrotetrazolium reduction, and ATP/DNA ratios. Appl. Environ. Microbiol. 51:150-156.

Jeffrey, W. H., and J. H. Paul. 1986. Activity measurements of planktonic microbial and microfouling communities in a eutrophic estuary. Appl. Environ. Microbiol. 51:157-162.

Jones, J. G., and B. M. Simons. 1975. An investigation of errors in direct counts of aquatic bacteria by epifluorescence microscopy, with reference to a new method for dyeing membrane filters. J. Appl. Bacteriol. 39:1-13.

Jordan, M. J., and G. E. Likens. 1980. Measurement of planktonic bacterial production in an oligotrophic lake. Limnol. Oceanogr. 25:719-732.

Karl, D. M. 1982. Selected nucleic acids precursors in studies of aquatic microbial ecology. Appl. Environ. Microbiol. 44:891-902.

Kirchman, D., H. Ducklow, and R. Mitchell. 1982. Estimates of bacterial growth from changes in uptake rates and biomass. Appl. Environ. Microbiol. 44:1296-1307.

Kornberg, A. 1974. DNA replication. W. H. Freeman and Company, San Francisco. 724 pp.

Laanbroek, H. J., and J. C. Verplanke. 1986. Seasonal changes in percentages of attached bacteria enumerated in a tidal and a stagnant coastal basin: relation to bacterioplankton productivity. FEMS Microbiology Ecology. 38:87-98.

Lancelot, C., and G. Billen. 1984. Activity of heterotrophic bacteria and its coupling to primary production during the spring phytoplankton bloom in the southern bight of the North Sea. Limnol. Oceanogr. 29:721-730.

Larsson, J., and A. Hagstrom. 1979. Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Marine Biology 52:199-206.

Lind, O. T. 1979. Handbook of common methods in Limnology. The C. V. Mosby Company, St. Louis. 199 pp.

Lovell, C. R., and A. Konopka. 1985. Primary and bacterial production in two dimictic Indiana lakes. Appl. Environ. Microbiol. 49:485- 491.

Lovell, C. R., and A. Konopka. 1985. Seasonal bacterial production in a dimictic lake as measured by increases in cell numbers and thymidine incorporation. Appl. Environ. Microbiol. 49:492-500.

Lovell, C. R., and A. Konopka. 1985. Thymidine incorporation by free-living and particle-bound bacteria in a eutrophic dimictic lake. Appl. Environ. Microbiol. 49:501-504.

Marshall, K. C. 1986. Advances in microbial ecology. Vol. 9. Plenum Press, New York. 240 pp.

Menzel, D. W., and R. F. Vaccaro. 1964. The measurement of dissolved organic and particulate carbon in seawater. Limnol. Oceanogr. 9:138- 142.

Moriarty, D. J. W. 1986. Measurement of bacterial growth rates in aquatic systems from rates of nucleic acid synthesis. Pages 245-292. In: Marshall, K. C. (ed),

Advances in Microbial Ecology. Vol. 9. Plenum Press, New York. 402 pp.

Moriarty, D. J. W., and P. C. Pollard. 1981. DNA synthesis as a measure of bacterial productivity in seagrass sediments. Mar. Ecol. Prog. Ser. 5:151-156.

Murray, R. E., and R. E. Hodson. 1985. Annual cycle of bacterial secondary production in five aquatic habitats of the Okefenokee Swamp ecosystem. Appl. Environ. Microbiol. 49:650-655.

Paerl, H. W. 1975. Microbial attachment to particles in marine and freshwater ecosystems. Microbial Ecol. 2:73-83.

Paerl, H. W. 1978. Microbial organic carbon recovery in aquatic ecosystems. Limnol. Oceanog. 23:927-935.

Paerl, H. W., and C. R. Goldman. 1972. Stimulation of heterotrophic and autotrophic activities of a planktonic microbial community by siltation at Lake Tahoe, California. Mem. Ist. Ital. Idrobiol. Suppl. 29:129-149.

Palumbo, A. V., R. L. Ferguson, and P. A. Rublee. 1984. Size of suspended bacterial cells and association of heterotrophic activity with size fractions of particles in estuarine and coastal waters. Appl. Environ. Microbiol. 48:157-164.

Parsons, T. R., Y. Maita, and C. M. Lalli. 1984. A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford. 173 pp.

Pedros-Alio, C., and T. D. Brock. 1983. The impact of zooplankton feeding on the epilimnetic bacteria of a eutrophic lake. Freshwater Biology. 13:227-239.

Peele, E. R., R. E. Murray, R. B. Hanson, L. R. Pomeroy, and R. E. Hodson. 1985. Distribution of microbial biomass and secondary production in a warm-core gulf-stream ring. Deep-Sea Research. 32:1393-1403.

Pollard, P. C., and D. J. W. Moriarty. 1984. Validity of the tritiated thymidine method for estimating bacterial growth rates: measurement of isotope dilution during DNA synthesis. Appl. Environ. Microbiol. 48:1076-1083.

Pomeroy, L. R. 1974. The ocean's food web, a changing paradigm. BioScience. 24:499-504.

Pomeroy, L. R. 1984. Significance of microorganisms in carbon and energy flow in marine ecosystems. Pages 405-411. In: Klug, M. J., and C. A. Reddy, (eds), Current Perspectives in Microbial Ecology. American Society for Microbiology, Washington. 710 pp.

Rheinheimer, G. 1985. Aquatic microbiology. John Wiley and Sons, New York. 257 pp.

Riemann, B. 1984. Determining growth rates of natural assemblages of freshwater bacteria by means of ^{3}H-thymidine incorporation into DNA: comments on methodology. Arch. Hydrobiol. Beih. Ergebn. Limnol. 19:67- 80.

Riemann, B., and S. Bosselmann. 1983. Daphnia grazing on natural populations of bacteria. Verh. Int. Ver. Limnol. 22:795-799.

Riemann, B., J. Fuhrman, and F. Azam. 1982. Bacterial secondary production in freshwater measured by the ^{3}H-thymidine incorporation method. Microbial Ecol. 8:101-114.

Riemann, B., and M. Sondergaard. 1984. Measurements of diel rates of bacterial secondary production in aquatic environments. Appl. Environ. Microbiol. 47:632-638.

Robarts, R. D., and L. M. Sephton. 1983. Heterotrophic activity and seasonal cycles of bacteria in a hypertrophic African lake (Hartbeespoort Dam, South Africa). Verh. Int. Ver. Limnol. 22:1204-1207.

Scavia, D., G. A. Laird, and G. L. Fahnenstiel. 1986. Production of planktonic bacteria in Lake Michigan. Limnol. Oceanogr. 31:612-626.

Simon, M., and M. M. Tilzer. 1982. Bacterial decay of the autumnal phytoplankton in Lake Constance. Schweiz. Z. Hydrol. 44:263-275.

SPSS, Inc. 1983. SPSS^{x} User's Guide. McGraw-Hill Book Co., New York.

Strickland, J. D. H., and T. R. Parsons. 1968. A practical handbook of seawater analysis. Fisheries Research Board of Canada Bulletin no. 167. Fisheries Research Board of Canada, Ottawa.

Toerien, D. F. 1985. Suspended sediment-microbial interactions in a shallow turbid impoundment. Verh. Internat. Verein. Limnol. 22:2726- 2731.

Van Es, F. B., and A. Meyer-Reil. 1982. Biomass and metabolic activity of heterotrophic marine bacteria. Pages 111-170. In: Marshall, K. C. (ed), Advances in Microbial Ecology. Vol. 6. Plenum Press, New York. 240 pp.

Wetzel, R. G. 1983. Limnology. Saunders College Publishing Co., Philadelphia. 767 pp.

Wolter, K. 1982. Bacterial incorporation of organic substances released by natural phytoplankton populations. Mar. Ecol. 7:287-295.

Wright, R. T. 1978. Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36: 297-305.