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# Distribution and dynamics of bacterioplankton production in a polymictic tropical lake (Lago Xolotlán, Nicaragua)

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**Abstract**

From 1988 to 1993 we assessed the variability of bacterioplankton production and biomass in Lake Xolotlán (L. Managua), Nicaragua via [$^3$H]thymidine incorporation into DNA and cell counting. Bacterial production ranged from 3 to 8 $\mu$g C $l^{-1}$ $h^{-1}$, and since production was equal throughout the water column, areal production was high ($\approx$ 600–1200 mg C $m^{-2}$ $d^{-1}$). Bacterial abundance in Lake Managua was extremely high, 7–30 $\times$ $10^9$ cells $l^{-1}$. Thus, specific rates of bacterial production were low. There was a strong correlation between production and number and the specific rate of bacterial production was constant. Comparable measurements of production via [$^3$H]leucine incorporation into proteins indicated that bacteria were experiencing 'balanced growth'. We conclude that bacterioplankton in Lake Xolotlán had reached its carrying capacity and a significant correlation between bacterial production and concentration of phaeophytin implied that dead or dying algae was the limiting substrate for bacterioplankton. The majority of bacterial number and most of bacterial production (up to 75%) were associated with particles in the $>$ 3-$\mu$m fraction, probably lysing algal cells to which bacterioplankton were 'attached'. Grazing on bacterioplankton must be low and bacteria should be a 'sink' for organic matter in Lake Xolotlán.

## Introduction

Pelagic heterotrophic bacterial production is about 30% of primary production, on an areal basis, across systems in both marine and freshwater environments (Cole et al., 1988). This relationship suggests either that bacteria and phytoplankton grow in response to common factors such as nutrient loading and temperature, or that phytoplankton or substances released by phytoplankton are important substrates for bacteria. There is generally a positive relationship between algal and bacterial biomass and increasing nutrient loading (Bird & Kalff, 1984), but the ratio of bacteria to algae is higher in oligotrophic lakes (Currie, 1990). In oligotrophic lakes, the paradoxical situation exists that bacteria compete directly with algae for inorganic nutrients (Currie et al., 1986), but are also dependent on the DOC released by algae (Bratbak & Thingstad, 1985). In such systems, algae are predominantly 'edible' forms (flagellates) and nutrients must be mainly recycled via grazing. With increasing eutrophication, 'inedible' algae (filamentous and colonial cyanobacteria etc.) predominate, a more indirect 'detrital' food web exists. If bacterial production is not grazed, bacteria will function more or less as a 'sink' for organic matter (Ducklow et al., 1986).

Since light and temperature are optimal, nutrient availability in tropical polymictic lakes can be of particular significance in controlling the level of production. High sustained primary production is a common phenomenon even when ambient inorganic nutrient concentrations are low or below detection levels (Viner, 1973), and this implies rapid nutrient cycling. Bacterial abundance in tropical lakes can exceed $10^{10}$ cells $l^{-1}$ (Rai, 1979; Rai & Hill, 1980), up to an order of magnitude higher than in temperate lakes (Bird & Kalff, 1984), but there is a paucity of information on bacterioplankton growth in the tropics (Gebre-Mariam & Taylor, 1989a, b).

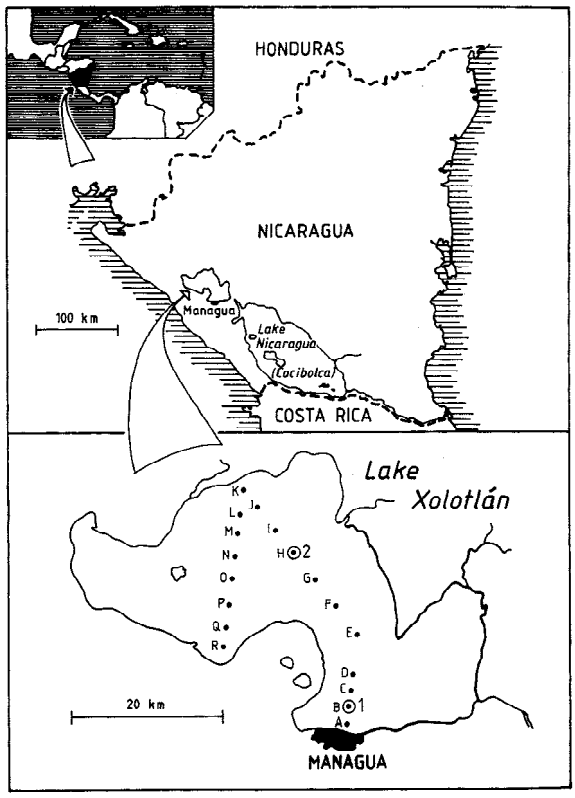


*Figure 1.* Nicaragua and Lake Xolotlán. 1 and 2 in the bottom panel are sampling stations in the southern and central basin. Transect sampling stations are marked with letters.

We have studied the bacterioplankton in tropical Lake Xolotlán, Nicaragua. In this lake algal biomass is dominated by 'inedible' forms (Erikson et al., 1997; Hooker et al., 1991) and we would expect bacterial mineralisation to be the main sink of organic matter. Phytoplankton primary production is high and stable (Erikson et al., 1997) and subsequently, this mineralisation by bacteria should be very efficient.

The geology and the physical and chemical properties of the lake have been described elsewhere (Erikson et al., 1997; Lacayo, 1991).

## Methods

### *Sampling regime*

Diel studies were performed in the central basin of Lake Xolotlán (station 2 [H]; Figure 1) on October 10–11, 1988, April 13–14, 1989, October 27–28, 1989, and April 3–4, 1990. Sampling was begun at 10 a.m., repeated at approximately 4 h intervals (depending on wind conditions) and ended with a final sampling at about 10 a.m. on the second day. Water was collected from discrete depths with a Van Dorn sampler. Thymidine incorporation was always measured at depths of 0.5 m, 3 m and 10 m, whereas leucine incorporation was measured at one or two depths. Only thymidine incorporation was measured during April 1990. Regular sampling and measurement of thymidine incorporation was performed from July 1988 to November 1993. This was done at two depths in the southern basin (station 1 [B]; Figure 1) at about 9 a.m. (20 times) and at three depths in the central basin (station 2 [H]; Figure 1) at about 10 a.m. (33 times). From November 1991 to October 1992 we made monthly transects of thymidine incorporation at two depths starting from the shoreline in the southern basin (stations A to E + H; Figure 1). Measurements along transects were also performed at two depths in the central basin (stations F to R; Figure 1) in December 1991 and February 1992.

Immediately upon collection of samples, lake water was filtered through 3-$\mu$m and 1-$\mu$m pore cellulose acetate membrane filters (Schleicher & Schuell), under low pressure (manual pump), and with successive changes of filters when pores became clogged. Three-$\mu$m size fractions were done with water from 0.5 and 10 m of all diurnal samplings and seven monthly samplings in 1992. One-$\mu$m size fractions were done on 3-$\mu$m-prefiltered water from 0.5 m of the two final diurnal samplings and the seven monthly samplings. Subsamples of filtered water were treated in the same way as samples of unfiltered water (see below).

Samplings for chlorophyll-*a*, phaeophytin, particulate and dissolved organic carbon and primary production were performed simultaneously with sampling for bacterioplankton and are described in Erikson et al. (1998).

### *Analytical procedures*

Bacterial abundance and cell size were assessed using epifluorescence microscopy (Hobbie et al., 1977). Samples for the enumeration of bacteria were placed in 20 ml plastic scintillation vials containing filtered formalin (4% final conc.). Due to high densities of colonial cyanobacteria, sonication was required before enumeration. This required two different treatments – one filter stained with acridine orange for total bacteria, and a second filter without acridine orange for the enumeration of cyanobacteria from autofluorescence. 0.1–0.2 ml of sample was diluted to $>2$ ml with sterile

tap water, sonicated 1 min in an ice bath with a Rapids 350 Ultrasonic Disintegrator at 20 kHz and 100 W, added to a Millipore funnel and mixed with an equal amount of 0.01% acridine orange or an equal amount of sterile tap water. The samples were filtered onto black 25 mm, 0.2-$\mu$m pore Nuclepore polycarbonate filters and examined using a Nikon Labophot fluorescent microscope and filter set G2-A for total bacteria or filter set G1-B for autofluorescence. Bacteria were counted in all samples from the diurnal study of October 1989 and in selected samples on other occasions. An extremely high abundance of bacteria in the samples of April 1990 made counting difficult, until we increased the dilution of subsamples up to 200:1 (valid for 10 m samples). Randomly selected bacteria were measured with an eyepiece micrometer and cell volumes were estimated by assuming standard geometric forms.

Assays for thymidine and leucine incorporation were made in 20 ml glass scintillation vials. Triplicate 5 or 10 ml samples and a formalin killed blank (2% final concentration) were incubated immediately at in situ temperature with 25 nM [methyl-$^3$H]thymidine (50 Ci mmol$^{-1}$; Amersham) or 10-100 nM L-[4,5-$^3$H]leucine (140 Ci mmol$^{-1}$) for no longer than 30 min. The optimum condition for thymidine incubation was chosen after preliminary experiments carried out in August 1988, which showed similar incorporation rates in incubations with 15, 25, 35 and 50 nM (CV = 8.2 and 12.2% at sites 1 and 2, respectively) and a linear increase of incorporation rates during the first hour of incubation ($n = 4$, $p < 0.01$ at both sites). The optimum condition for leucine incorporation was determined from experiments conducted in April 1989, which showed a typical unsaturated uptake with less than 25 nM leucine, and therefore the leucine incorporation rates from October 1988 (10 and 20 nM) may be underestimated, especially when only 10 nM of leucine was added. The incubations were stopped by adding formalin to a final concentration of 2% and the vials were immediately placed in an ice chest, then in a refrigerator (<4°C) upon return to the laboratory. Filtrations were begun within several hours after return to the laboratory.

The extraction and filtration procedure for estimating incorporation into macromolecules (material insoluble in cold trichloroacetic acid [TCA]) was similar for both isotopes. 1 ml of ice-cold 40% TCA was added to 5 ml subsamples. After about 15 min the samples were filtered onto 25 mm diameter, 0.45-$\mu$m pore cellulose acetate membrane filters (Schleicher & Schuell OE 67) using a 10-place filtration unit. The stainless steel funnels were kept cold between uses. After 5 × 1 ml rinses with cold 5% TCA, the filters were also rinsed with 5 x 1 ml portions of cold 80% ethanol (Robarts & Wicks, 1989; Wicks & Robarts, 1987). Radioactivity on filters was measured with an LKB Model 1217 Rackbeta liquid scintillation counter. The coefficient of variation for triplicate determination averaged 12%.

*Data handling*

Incorporation rates of thymidine was converted to bacterial carbon production according to Bell et al. (1983):

g C l$^{-1}$ h$^{-1}$ =

(moles of [$^3$H]thymidine in cold TCA insoluble extract· conversion factor [cells mol$^{-1}$] · average cell carbon · 60)/incubation time [min]

Average cell carbon was determined from the carbon volume$^{-1}$ vs. cell size relationship derived by Simon & Azam (1989) [30 fg cell$^{-1}$ for 0.2 $\mu$m$^3$]. Production of bacterial cells was calculated using the empirical conversion factor of $2{\cdot}10^{18}$ cells mol$^{-1}$ (Bell et al., 1983; Riemann & Bell, 1990; Smits & Riemann, 1988; Riemann) derived from several eutrophic freshwater environments.

Incorporation rates of leucine was converted into bacterial carbon production (g C) as: (mol leu inc.) · 1797 · 0.86 · ID (Simon & Azam, 1989), where 1797 is derived from the molar percentage of leucine in protein (7.3%) and the mol weight of leucine (131.2) and 0.86 is the C:protein ratio. ID is the isotope dilution factor and ranges from 2 to 4 (Riemann et al., 1990; Simon & Azam, 1989). We used an ID of 4 which makes the leucine dilution equal to the approximate thymidine dilution that is included in the empirical conversion factor above (Bell, 1990; Riemann & Bell, 1990; Riemann et al., 1990). In conservative estimates ID is assumed to be 1 for leucine. This is true also for thymidine and a corresponding theoretical conversion of incorporation rates of thymidine into conservative estimates is accordingly based on the molar percentage of thymidine in DNA (25%), the mol weight of thymidine (318; nucleotide average) and the C:DNA ratio. However, whilst the C:protein ratio is constant for all cell volumes, the C:DNA ratio is cell volume dependent (Simon & Azam, 1989). Therefore, we relied on the empirical conversion factors for thymidine

incorporation and used the theoretical conversion only as a control.

[$^3$H]thymidine incorporation into DNA and [$^3$H]leucine incorporation into proteins as percentages of total incorporation into macromolecules were approximated to 80 and 90%, respectively (Riemann & Bell, 1990).

## Results

### *Temporal variability*

Diel measurements comprised four 24-h periods during the two seasonal extremes; the end of the dry and the end of the rainy season (Figure 2). Averages (and SD) of bacterial production of the diel cycles were 92 ± 33, 75 ± 16, 47 ± 14 and 134 ± 37 pmol [$^3$H]-thymidine $l^{-1}$ $h^{-1}$, respectively (in chronological order). Only during the first diel cycle (Oct. 1988) was the CV greater than 30%, mainly as a result of high rates during the first half of the diel cycle and decreasing rates during the final part. Averages of bacterial abundance were 19 ± 1.5, 12 ± 1.5, 6.2 ± 1.0 and 26 ± 7.1 × $10^9$ cells $l^{-1}$, respectively. Bacterial numbers were significantly lower in surface waters than in bottom waters in April 1990, which could be an artefact due to methodological problems with the dilution of subsamples for counting (see Methods). The cell volume was on average 0.2 $\mu m^3$ during all periods. Generally, each diurnal period represented distinct levels of both bacterial production and abundance and no specific seasonal resemblance's are evident. Nor could any general diurnal trend be discerned. The CV for all hourly depth averages of rates and abundance's was 3% and 10%, respectively.

We have records from every second day of sampling in series comprising one week in the southern basin (Oct. 1988) and four weeks in the centre of the lake (Oct. 1988 and 1989, April 1989 and 1990). Variation of bacterial production within a week was small in the southern basin (CV = 10%), but somewhat higher in the centre (CV = 22–29%; Figure 3b). Infrequent counts of bacteria indicated a similar variability of bacterial number in both basins. We also have records of bacterial production from monthly samplings during a year in the centre of the lake (November 1991 to October 1992), that show a similar moderate variation (CV = 26%; Figure 3B). These data indicated a distinct seasonality in bacterial production over the year; bacterial production increased during the dry season and decreased during the rainy season. Data from other years are, however, not frequent enough to confirm if this is a true intraseasonal pattern or not, and bacterial production is generally not predictable from one season to another (see Figure 2).

The variability of bacterial production over years was more significant (CV > 40%) and coincided between basins (Figure 3a, b). Bacterial production was relatively low until 1990 and thereafter considerably higher until becoming low again in late 1993. Averages of bacterial production, as reflected by incorporation of [$^3$H]thymidine (pmol $l^{-1}$ $h^{-1}$), during these two distinct periods was 82 ± 20 ($n$ = 8) and 173 ± 65 ($n$ = 11) in the southern basin and 59 ± 19 ($n$ = 12) and 118 ± 29 ($n$ = 12) in the centre of the lake. The variability of bacterial number in the centre of the lake (Figure 3c) coincided with the temporal shifts in bacterial production.

Measurements of other parameters were also performed over yearly and diurnal cycles. Long term variations of chlorophyll-*a*, phaeophytin, particulate and dissolved organic carbon (POC, DOC) in the centre of the lake are shown in Figure 3d–g. Only DOC concentration demonstrated low variability (CV = 18%). During the diurnal cycles variability was low (CV < 15%) for all parameters (Erikson et al., this volume). Daily gross phytoplankton production, measured as both oxygen evolution and $^{14}$C assimilation, was high (6–7 g C $m^{-2}$ $d^{-1}$) and stable over temporal and spatial scales (CV = 10%) and phytoplankton cell growth, measured as the difference between incorporation rates of adenine and thymidine, was 4–5 g C $m^{-2}$ $d^{-1}$ (Erikson et al., 1998).

### *Spatial variability*

Differences between the central lake and the southern basin, expressed as mean ratios between all simultaneously measured bacterial production rates and bacterial numbers at the two sampling sites, were about 0.7 for both parameters (CV < 30%; data from Figure 3). Transect samplings of the two basins (Figure 4a, b) were performed monthly during an entire year (1992). Horizontal variation within basins was small and bacterial production and numbers were significantly different only near the shore or close to inputs of DOM. For example, bacterial production and numbers just in front of the city of Managua reached extremely high annual averages of 317 ± 114 pmol [$^3$H]-thymidine $l^{-1}$ $h^{-1}$ and 50 · $10^9$ ± 14 · $10^9$ cells $l^{-1}$ (only 0.5 m samples). For most parts of the lake

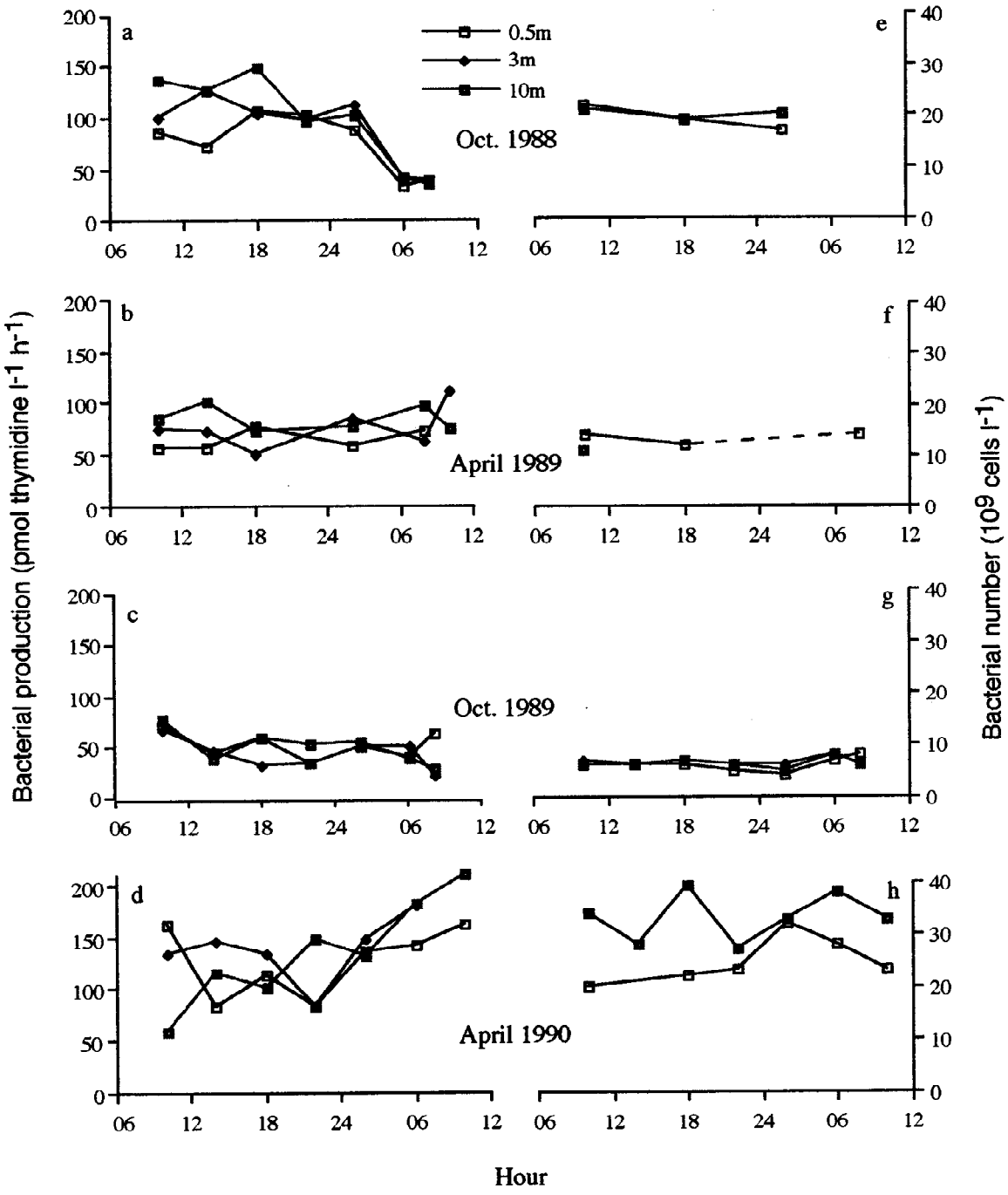


*Figure 2.* Diurnal variation of bacterial production (left panel) and bacterial number (right panel) in the central basin. From top tobottom; 10–11 October 1988, 13–14 April 1989, 27–28 October 1989 and 3–4 April 1990.

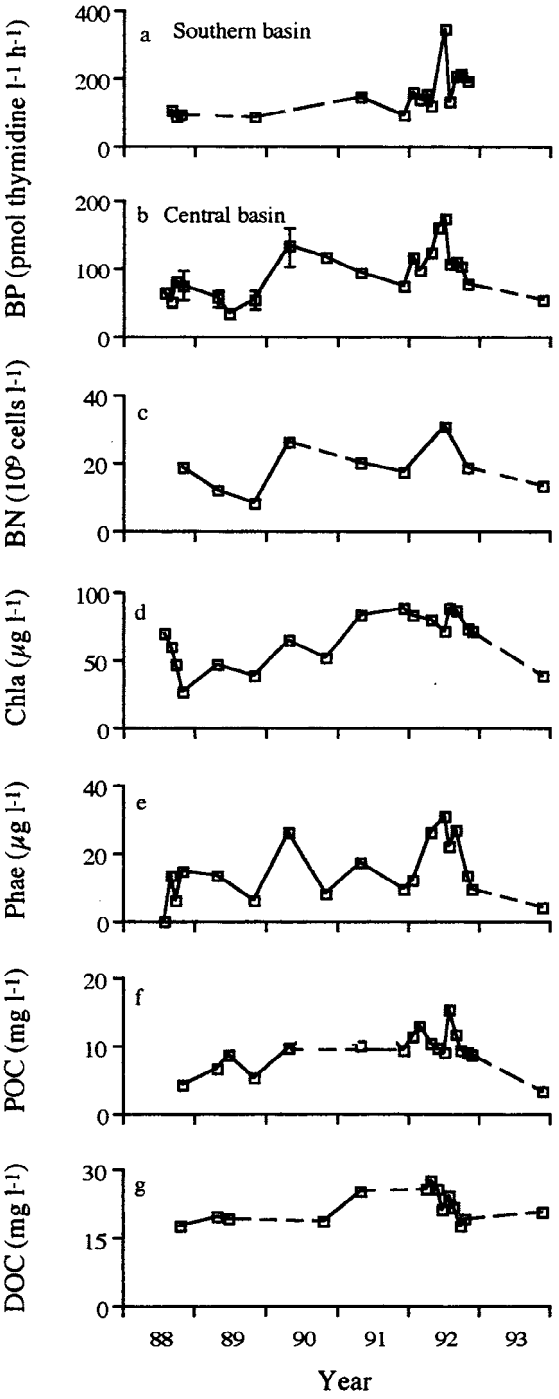


*Figure 3.* Long-term variations of (a) bacterial production (BP) in the southern basin and (b) bacterial production (BP), (c) bacterial abundance (BN), (d) chlorophyll-*a* (Chla), (**e**) phaeophytin (Phaeo), (f) particulate organic carbon (POC) and (g) dissolved organic carbon (DOC) in the cental basin. All data are water column averages (mostly only two depths for BN) from the 10 a.m.samplings. Some data are also averages of records from adjacent days. Standard deviation (SD) is inserted to show the variation within such bacterial production data. Only data on Chla, Phaeo and POC parallel to BP and BN are accounted for here, more data are presented in Erikson et al. (1998).

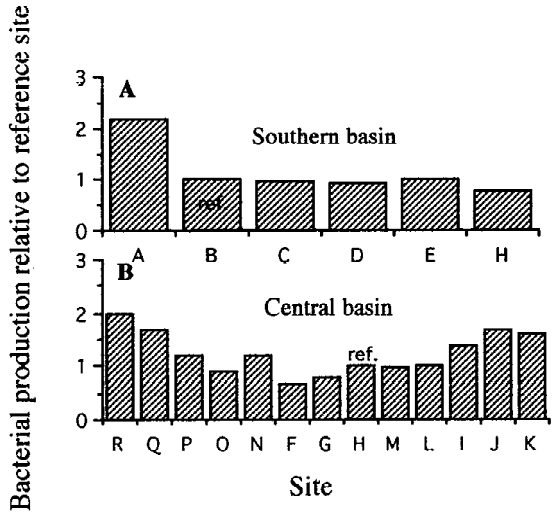


*Figure 4.* Bacterial production from monthly sampling (1991–1992) of transects in (a) southern basin and (b) central basin, with sampling sites arranged according to depth and position within basins (see Figure 1). The bacterial production at each sampling site is expressed as a yearly water column average value relative to a reference station, which was the regular sampling sites 1 (B) in the southern basin and 2 (H) in the central basin.

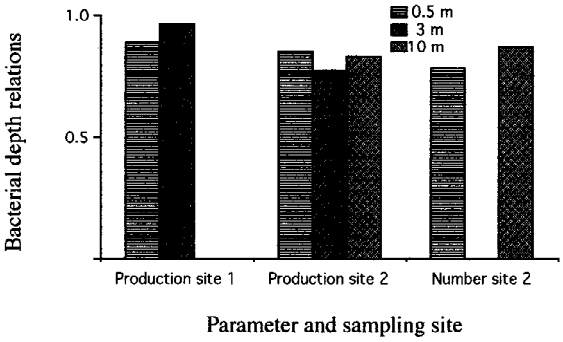


*Figure 5.* Bacterial production and number in different depths of the water column in the southern and central basin. Highest vertical rate at each daily occasion (10 a.m.) was given a value of unity and rates at the other depths values proportional to this. The figure is composed of the averages of all such measurements from the long term sampling serie (see Figure 3a–c).

however, regular sampling sites 1 and 2 could be considered as representative of their respective basin. The CV for rates in the southern basin (A excluded) was negligible and 25% for numbers in the southern basin (only 0.5 m samples; A excluded) and 23% for rates in the central basin (sites close to shore excluded).

The differences with depth of bacterial production and number during the long term sampling (see Figure 3a–c) were small (average CV $< 20\%$; Figure 5). Cell counts from the water column at site 1, although

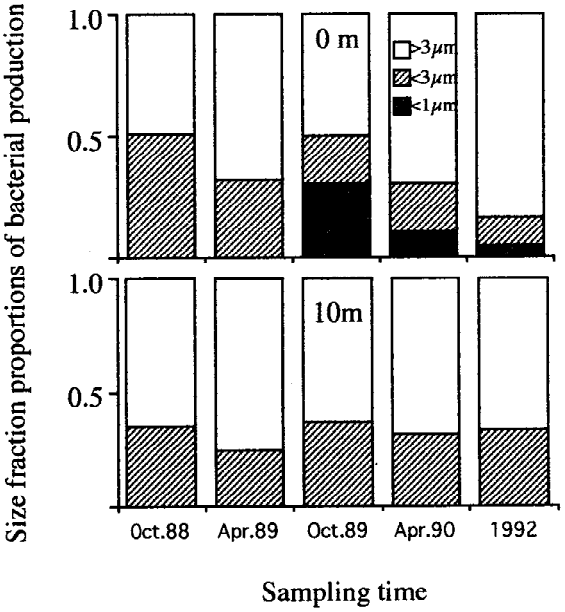


*Figure 6.* Proportions of bacterial production in different size fractions. Data are from two depths of the central basin and averages of four diurnal and one monthly sampling serie.

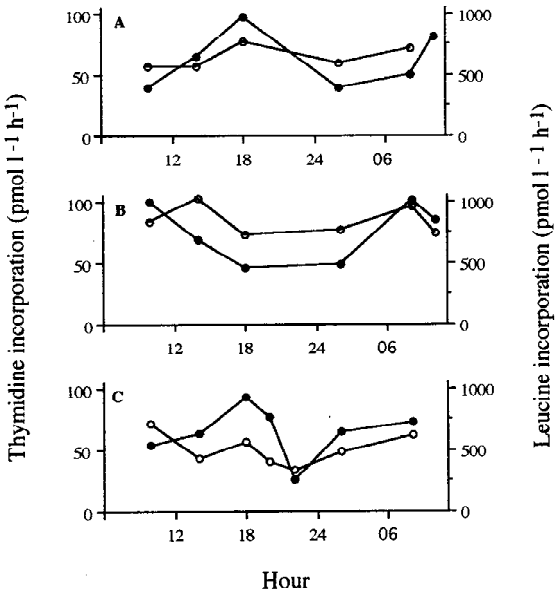


*Figure 7.* Diel variation of bacterial production measured as incorporation of thymidine (○) and leucine (●). Data from (a) 0.5 m in April 1989, (b) 10 m in April 1989 and (c) 0.5 m in October 1989.

less frequent, indicated that this was true also for the depth variations of bacterial number in the southern basin. Additionally, there were no great differences in bacterial production and number between depths during the diurnal sampling periods (see Figure 2).

*Size fractions*

Bacterial production rates within different size fractions are expressed as proportions of corresponding total rates (Figure 6). During the diurnal cycles the greatest proportion of the bacterial production within the <3-$\mu$m fraction was found in the surface water by the ends of the rainy seasons (Oct. samplings), but it was still not higher than 0.5. By the ends of the dry seasons (April samplings) the proportion was lower and varied less with depth. Proportions of bacterial cell numbers within the <3-$\mu$m fraction showed a similar pattern. We have a complete set of counts from October 1989, which show proportions within the <3-$\mu$m fraction of 0.59 (CV = 16%) and 0.47 (CV = 21%) of surface and deep water bacteria, respectively. Infrequent counts from the dry periods demonstrated even lower corresponding proportions. The proportion of bacterial production within the <1-$\mu$m fraction on April 1990 was also lower than that on October 1989. The distribution of bacterial production within the different size fractions during 1992 resembled that of the dry seasons, especially that of April 1990. Rain was scarce during 1992 and the entire year could be classified as a drought (Erikson, 1998). Thus, on average during rainy seasons the proportions of bacterial production within the <3-$\mu$m size fraction were 0.50 (CV = 21%) in the surface water and 0.64 (CV = 29%) in the deep water, whilst during dry seasons the corresponding proportions were 0.74 (CV = 15%) and 0.70 (CV = 26%).

*Protein synthesis*

At the same time as the measurements of incorporation of [$^3$H]thymidine (production as DNA synthesis) were made, we also measured the incorporation of [$^3$H] leucine (production as protein synthesis) in four diurnal series of sampling (0.5 m in Oct. 1988 and 1989, 0.5 and 10 m in April 1989) and in some <3-$\mu$m fraction samples (April 1989). Measurements in October 1988 gave extremely low rates of [$^3$H] leucine incorporation, which could be the result of using a lower concentration of leucine on those occasions (10 nM, see methods), and these data are neglected. On the other occasions results of the two methods covaried during the diurnal cycles (Figure 7) and generally bacteria synthesised DNA and proteins at proportional rates, although protein synthesis tended to be more variable than DNA synthesis over a 24-hour period

(average CV = 34% and 17%, respectively). Incorporation rates of [$^3$H]thymidine and [$^3$H]leucine covaried also in the <3-$\mu$m samples but DNA synthesis was much higher relative to protein synthesis compared to what it was in the unfiltered samples.

*Calculations and relationships*

According to our formula for the conversion of thymidine incorporation rates and with correction for the fraction bound into macromolecules other than DNA (see Methods) bacterial production in Lake Xolotlán during 1988–1992 ranged from 2.8 to 5.7 $\mu$g C $l^{-1}$ $h^{-1}$ in the central basin and 3.9 to 8.3 $\mu$g C $l^{-1}$ $h^{-1}$ in the southern basin. During the year 1991-1992 it averaged 15.3 $\mu$g C $l^{-1}$ $h^{-1}$ in the littoral zone in front of the city of Managua. Seasonal averages of diurnal bacterial production, assuming equal rates during day and night (see Figure 2), are presented in Table 1. On an areal basis, calculated for the average depth of each basin and period (considering water level variations; Erikson, this volume) bacterial production was approximately 700–1200 and 600–800 mg C $m^{-2}$ $d^{-1}$ in the central and southern basins, respectively. Ranges within and differences between basins thus decreased when transforming bacterial production from volume to areal rates. Areal bacterial production was also higher in the central basin compared to the southern basin. Bacterial biomass in the central basin ranged from about 0.2 to 0.9 mg C $l^{-1}$ (based on an average cell volume of 0.2 $\mu m^3$) and was 4–13% of total particulate organic carbon (POC; Table 1).

Specific bacterial production (production per biomass) is an index of bacterial specific growth rate and is usually expressed as thymidine incorporation per cell. We have no information on the number of inactive cells, but provided that they were few, the specific bacterial production in central Lake Xolotlán averaged 4.7 $\times$ $10^{-21}$ mol $cell^{-1}$ $h^{-1}$ and was stable (CV = 17%) throughout seasons and for different biomass (POC) concentrations (Table 1). Thus, bacterial production and biomass were significantly correlated ($r^2$ = 0.90; $p < 0.001$) and the variation of the bacterial biomass was the main factor determining bacterial production. An equally strong correlation between bacterial production and number ($r^2$ = 0.96) was evident from the transect data (only 0.5 m samples). Phaeophytin concentration was the external factor best correlated to bacterioplankton production and biomass ($r^2$ = 0.56 and 0.78, respectively; $p < 0.01$).

## Discussion

*Patterns of variation*

The variability of bacterial production and abundance can be summarised as follows: There were generally no vertical differences (CV < 20%). Variation during short-term time scales (hours and days) was low (CV < 30%). This was also true for variations within a year or within the lake, although certain trends were distinguishable at each scale; a coupling to seasonal events and to bathymetric conditions (see below). Significant differences (CV > 40%) were apparent only during longer time scales (years) and for extreme localities (polluted littoral zones). The constant climate and the complete mixing within the lake are factors that promote the low variability of bacterioplankton in the polymictic Lake Xolotlán. Other studies of nutrients, phytoplankton and primary production in Lake Xolotlán confirm the low temporal and spatial variability (Erikson et al., 1997). In stratified temperate lakes, on the other hand, rates of thymidine incorporation are often at least an order of magnitude greater in the upper epilimnion (Bell et al., 1983) than in the hypolimnion. In mesotrophic Lake Erken, Sweden, rates of thymidine incorporation in the epilimnion ranged from 2 to 50 pmol $l^{-1}$ $h^{-1}$ during summer stratification (Bell, 1984).

Variability, beside that over years and gradients of pollution (DOM), did exist and was related to bathymetric and seasonal conditions. For example, bacterial production was enhanced over shallower stations (Figure 4). This might suggest the importance of sediment resuspension in stimulating bacterial production in the water column (Wainwright, 1987). However, sediment microbial activity was only 6% of the total heterotrophic activity (Ahlgren et al., 1997), and the resuspension of sediments would not contribute much to the pelagic bacterial production. Instead, just as phytoplankton biomass per unit volume was higher in shallower parts, but more or less uniform per unit area over the lake (Erikson et al., 1997), this sort of distribution could be a general pattern for bacterioplankton as well. Wind induced horizontal translocation of water masses could disrupt this distribution (Erikson, 1998), which might explain the high bacterial production in the centre of the lake during the first part of the diel cycle in October 1988. The preceding night had been stormy, but the day of sampling was calm until wind and mixing started in the late afternoon. Such dramatic changes can occur during the rainy season

*Table 1.* Bacterial production, bacterial biomass, particulate organic carbon (POC) and specific bacterial production in the central basin from clearly defined periods. All data from Figure 3

| | | Bacterial production ($\mu$g C l$^{-1}$ d$^{-1}$) | Bacterial biomass ($\mu$g C l$^{-1}$) | POC (mg l$^{-1}$) | Specific production ($10^{-21}$ mol cell$^{-1}$ h$^{-1}$) |
|---|---|---|---|---|---|
| October | 1988 | 84 | 570 | 4.4 | 3.9 |
| April | 1989 | 65 | 360 | 6.6 | 4.7 |
| October | 1989 | 62 | 240 | 5.5 | 6.6 |
| April | 1990 | 152 | 780 | 9.7 | 5.1 |
| April | 1991 | 106 | 600 | 10.4 | 4.6 |
| November | 1991 | 98 | 510 | 9.4 | 4.9 |
| June | 1992 | 200 | 930 | 9.0 | 5.6 |
| November | 1992 | 88 | 570 | 9.0 | 4.1 |
| November | 1993 | 63 | 410 | 3.5 | 4.1 |

and cause sudden changes in the water column. During the dry season (April samplings) weather conditions are more predictable with constant strong winds and mixing.

*Attached bacteria*

Attached bacteria are by our definition those retained on a 3-$\mu$m filter. There is no microscope study to confirm this, because all samples were sonicated. The proportion of attached bacteria and associated bacterial production in Lake Xolotlán was much higher than has been found in many other lakes. In temperate lakes the proportion of total bacteria attached to particles is generally less than 10% (Kirchman, 1983; Kirchman & Mitchell, 1982), and in other warm and tropical lakes the proportion of attached bacterial number and productivity has been found to be less than 35% (Berman & Gerber, 1980; Gebre-Mariam & Taylor, 1989a, b; Lewis et al., 1986). In Lake Xolotlán about 50% of bacterial number and up to 75% of bacterial production was bound to particles larger than 3-$\mu$m (Figure 6). According to the strong correlation between POC and chlorophyll-*a* ($p < 0.001$; Erikson et al., this volume), organic particles in that size fraction were mostly of phytoplankton origin. Consequently, a much higher proportion of bacteria in Lake Xolotlán was attached to algal cells than is the case for many other lakes. Furthermore, as demonstrated by proportions in cell number versus production (see above), attached bacteria were relatively more productive than free bacteria. Thus, some sort of specific advantage for bacterioplankton in attaching to algae over living free must exist in Lake Xolotlán. Total bacterial number and production were correlated to phaeophytin (see above). The concentration of phaeophytin was high in Lake Xolotlán (average of this study =14 $\mu$g l$^{-1}$) and may be considered an indicator of algal mortality. A greater number of dead or dying algae liable to bacterial attack may be the specific factor favouring bacterial attachment in Lake Xolotlán.

*Balanced growth*

The average of arithmetic ratios between the leucine and thymidine uptake rates, corrected for uptake into macromolecules other than DNA (see methods), was 10.8 (CV = 28%). According to the conversions of incorporation rates to carbon production, a ten-fold difference between incorporation rates of leucine (when ID = 4) and thymidine will give the same production of carbon (see Methods). Thus, incorporation rates of [$^3$H]thymidine and [$^3$H] leucine in Lake Xolotlán did not only covary, they also gave similar estimates of the bacterial carbon production. This agreement between the two methods is of course a result of our choice of an ID factor for leucine of 4, which we justify only by the conclusion on theoretical grounds that the empirical conversion factor for thymidine ($2 \cdot 10^{18}$ cells mol$^{-1}$) also contains a dilution factor of about 4 (Bell, 1990). ID for leucine could just as well be differently. However, incorporation rates of both [$^3$H]thymidine and [$^3$H] leucine, when converted into conservative estimates (see Methods), will also give about the same bacterial carbon production in Lake Xolotlán, if C:DNA is about 20. Such a C:DNA ratio is not unlikely when cell volume is approximately 0.2 $\mu$m$^3$ (Riemann et al., 1990). The leucine:thymidine ratio

of arithmetic averages from measurements in the <3-$\mu$m fractions remained, however, low (= 5.9; CV = 11%), indicating that free living bacteria behaved differently. As the proportion of free bacterial production was variable in time and depth and differed significantly between April and October 1989 (Figure 6), different ratios for free and attached bacteria might explain some of the remaining discrepancies between the thymidine and leucine incorporation rate curves in Figure 7.

The coupling between DNA synthesis and protein synthesis, the constant growth rate and the low short-term variability, demonstrate that attached bacterioplankton in Lake Xolotlán were experiencing 'balanced growth'; cell division (DNA synthesis) and cell metabolism (protein synthesis) were in balance. Most of the bacterial abundance and productivity was associated with algal cells, on which bacteria grow slowly at a constant rate. In contrast to this, free living bacteria showed an enhanced DNA synthesis and a 'shift down' in metabolism. An explanation may be that when an individual bacterium is ready for cell division it separates from the algae or when separated, it takes the opportunity to divide, meanwhile searching for a new algae to exploit.

*Conversions to carbon*

Carbon conversion factors can be evaluated by comparing the two methods ([$^3$H]thymidine and [$^3$H] leucine incorporation). If we believe that both methods measure de facto bacterial production, covariation between results must imply that they measure the same bacterial production under 'balanced' conditions. Conversion factors are to be corrected according to that. Thus, we have converted incorporation rates of [$^3$H]thymidine and [$^3$H] leucine into carbon production either correctly or incorrectly in both cases. Or, in other words, in relation to true bacterial production, the degree of bias is equal in both estimates, and our conversion of [$^3$H]thymidine incorporation rates into carbon production would probably be less accurate if we where to chose other empirical factors, for example the factor of $1 \cdot 10^{18}$ cells mol$^{-1}$ used by Moriarty (1986). However, a C:DNA ratio of about 20 (see above), and a DNA content per cell of 2.5 fg (Riemann et al, 1990), would give a value of approximately 50 fg C per bacterial cell in Lake Xolotlán. This value is higher than both the factor of 30 fg C cell$^{-1}$ (see Methods) that we have used and the factor of 44 fg C cell$^{-1}$, that is the result of a widely accepted empirical factor of 220 fg C per 1 $\mu$m$^3$ (Bratbak & Dundas, 1984). Thus, we have probably not overestimated production and biomass of the bacteria in Lake Xolotlán.

*Lake comparisons*

We have compared our data from Lake Xolotlán to corresponding data from some other eutrophic and tropical lakes (Table 2). Rates in Lake Xolotlán were high, but not extremely high. Unlike temperate and stratified lakes, however, bacterial production in Lake Xolotlán remained high during the whole year and throughout the whole water column. On an areal basis, bacterial production was higher than in any other system included in the analysis of Cole et al. (1988). Bacterial production on an areal basis was nonetheless 20–30% of that of phytoplankton (Erikson et al., this volume), being on the low side of the cross-system average (median 27%) found by Cole et al. (1988). Considering the constant high temperature and constant input of DOM, an even higher bacterial production in Lake Xolotlán might be expected, especially in the southern basin, but impacts of these factors on bacterial production were seen only close to shore, elsewhere effects were reduced. Thus the major influence of the pollution from the city seemed localised to a narrow belt. The abundance's of pathogenic bacteria also decreased rapidly with distance from the shore (Vargas, et al. 1991).

What is really striking in the comparison with other lakes is the extremely high abundance of bacteria cells found in Lake Xolotlán. Converted to carbon, the bacterial biomass was several times higher than the maximum value reported by Riemann & Søndergaard (1986) for eutrophic lakes, and its proportion to pelagic carbon (POC) was also high. For example, in eutrophic Lake Norrviken, where POC was similar to Lake Xolotlán, bacterial biomass was less than 0.1 mg C l$^{-1}$ (Bell et al., 1983), i.e. only about 10% of that in Lake Xolotlán. The significance of bacterioplankton in Lake Xolotlán thus becomes most apparent on a biomass basis.

The bacterial biomass and the bacterial production, corrected for the thymidine uptake into macromolecules other than DNA, gives a turnover time for the bacterial community in Lake Xolotlán close to one week (see Table 1). Other studies in both marine and freshwater systems have presented evidence for much more rapid turnover times (0.2–2 d) of bacterial communities (Ducklow & Hill, 1985; Kirchman et al., 1982; Scavia & Laird, 1987). Also the specific

*Table 2.* Comparison of bacterial production rates and numbers in selected tropical and temperate eutrophic lakes

| Lake | Bacterial production ($\mu$g C l$^{-1}$ h$^{-1}$) | Bacterial number ($10^9$ cells l$^{-1}$) | Reference |
|---|---|---|---|
| Awassa, Ethiopia | 0.3 – 3.6 | 3.9 – 8.3 | Gebre-Mariam & Taylor (1989) |
| Cocibolca, Nicaragua | 2.7 | 4 – 10 | Erikson et al. (unpubl. data) |
| Xolotlan, Nicaragua | 2.8 – 8.3 | 7 – 30 | This study (no extreme data) |
| Chapala, Mexico | ≈1.0 | ≈10 | Chrzanowski et al. (1993) |
| Hartbeespoort Dam, S. Africa | 0 – 1.9 | 2.5 – 32 | Robarts & Wicks (1989) |
| Embalse del Rio 3, Argentina | 2.4 – 5.8 | 1 – 9 | Mariazzi et al. (1991) |
| Fredriksborg Slottsø, Denmark | 5.4 | 10 | Riemann & Søndergaard (1986) |
| Norrviken, Sweden | 0.2 – 7.1 | 0.5 – 2 | Bell et al. (1983) |

production of 4.7 $\times$ $10^{-21}$ mol cell$^{-1}$ h$^{-1}$ in Lake Xolotlán was extremely low. In Lake Norrviken, the specific production was ten times higher and the bacterial population was doubling 1–2 times per day (on the average) during the summer (Bell et al., 1983). Thus, despite the high water temperature, the turnover time in Lake Xolotlán was long and we conclude that the bacterioplankton were constantly in a 'stationary growth phase' (high abundance, slow turnover). This conclusion is supported by our observation of a balanced growth, because balanced growth is to be expected during a stationary growth phase (Kirchman et al., 1986). A stationary growth phase, with a tight coupling between production and biomass, a high carbon biomass and a constant low specific production, imply that bacterioplankton in Lake Xolotlán had reached its carrying capacity.

*Limiting substrate*

The carrying capacity for a bacterial population is reached during certain specific conditions. Wright & Coffin's (1984) model of bacterial dynamic interactions with substrate supply and grazing of bacteria predict that with high grazing rates, bacterial production approaches the maximum growth rate for the population and bacterial populations will be below the carrying capacity of the system. In the absence of predators the model predicts that bacterial growth is a function of the substrate supply. The maximum bacterial population achieved will be determined by the substrate concentration, specific production will be low and most of the substrate utilised by bacteria will be used for maintenance energy (Coffin & Sharp, 1987). On the basis on what we know about bacteria in Lake Xolotlán, Wright & Coffin's model predicts that grazing on bacteria was low and that bacterial number and production was limited by substrate supply.

We expect that limiting substrate for bacteria in Lake Xolotlán was some form of phytoplankton carbon, because effects of allochthonous organic carbon seems to be reduced to areas close to inputs of DOM. Primary production, DOC/EDOC and chlorophyll-*a* are resources to which rates and numbers of heterotrophic bacterioplankton often relate (Bratbak & Thingstad, 1985; Larsson & Hagström, 1979, 1982). In Lake Xolotlán primary production was temporally stable (Erikson et al., this volume), as was concentration of DOC ( CV = 18%). These could certainly compose the basic resources for bacterioplankton in Lake Xolotlán, but, in the absence of any covariation, we believe that they did not regulate it as limiting substrates. Chlorophyll-*a* varied with time and bacterioplankton were also within the 95% confidence range predicted from chlorophyll-*a* using the regression of Bird & Kalff (1984), but specific covariation do not appear in our material. Thus, lysing algal cells, as indicated by phaeophytin, remain as the only carbon source distinctly correlated to bacterioplankton. Robarts & Wicks (1990) also demonstrated a coupling between concentration of phaeophytin and bacterial production in another warm eutrophic lake, Hartbeesport Dam in South Africa, where concentrations of phaeophytin were just as high as in Lake Xolotlán (average $\approx$ 16 mg m$^{-3}$; our estimates from their data).

The concentration of phaeophytin is an indicator of dead or dying algae and the proportion of photosynthetic pigments to algal carbon biomass ($\approx$ 1:100; Erikson et al., 1998) shows that the supply of carbon

from dead algae available as substrate for bacterioplankton was considerable (on average $\approx$ 1.5 mg $l^{-1}$). The enhanced algal mortality in Lake Xolotlán is caused by respiratory losses in the dark portion of the water column (Erikson, 1998), which explains why areal bacterial production is higher in the central basin despite the higher degree of eutrophication in the southern basin, because relatively more algae are bound to die in the deeper part of the lake (Erikson, 1998).

In conclusion, bacterioplankton dynamics in Lake Xolotlán was regulated only by the amount of lysing algal cells, on which bacteria grew slowly at a constant rate. The bacterial biomass was therefore extremely high and the specific production and turn over rate accordingly low, which imply that the bacterioplankton was a 'sink' for carbon in the lake.

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