THE RELATIONSHIP BETWEEN BACTERIAL HETEROTROPHIC ACTIVITY AND ALGAL AUTOTROPHIC ACTIVITY IN EMBALSE DEL RIO III RESERVOIR (CORDOBA, ARGENTINA)

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RESUMEN: **Relación entre la actividad heterotrófica bacteriana y la actividad autotrófica algal en el Embalse del Río III (Córdoba, Argentina).**

La actividad autotrófica del fitoplancton se estimó mediante la técnica de Steemann Nielsen (1952) y la actividad heterotrófica del bacterioplancton mediante el cálculo de los parámetros cinéticos para ^{14}C-glucosa (Wright & Hobbie, 1965) y el consumo anaplerótico de CO_2 según Overbeck (1981). La máxima actividad heterotrófica fue observada en la zona eufótica (833 $\mu g\ C\ l^{-1}\ h^{-1}$) en coincidencia con la mayor tasa de producción primaria (338,7 mg $C\ m^{-2}\ d^{-1}$) y numerosidad de *Peridinium gatunense* (fin de verano). El potencial heterotrófico calculado en base al consumo de glucosa, comprende solo una pequeña parte del proceso de degradación total de la materia orgánica disuelta. El 11% del carbono fijado fotosintéticamente fue descompuesto por las bacterias como C-glucosa. Durante el verano se observó una mayor afinidad de las bacterias heterotróficas por la glucosa en la zona eufótica, mientras que en los meses de invierno se detectó una comunidad fisiológicamente diferente, con mayor afinidad por otros compuestos orgánicos, siendo la relación C-glucosa/C-CO_2 inferior a la unidad. En la composición fitoplanctónica se observa dominancia estival de dinoflagelados e invernal de diatomeas.

INTRODUCTION

To understand the dynamics of processes mediated by heterotrophic bacteria their metabolic activity must be known. There are various methods for measuring metabolic activities (oxygen consumption, CO_2 production, CO_2 dark fixation, determination of the kinetic parameters of organic substrates uptake, incorporation of ^{3}H-thymidine, etc.) Due to the complexity of natural substrates, the estimation of the heterotrophic potential based on only one of them (eg. glucose) is not always sufficiently representative of the heterotrophic activity as a whole. According to Overbeck (1980) and Cavari *et al.* (1979), the heterotrophic activity calculated from Vmax for glucose in PluBsee and in Lake Kinneret, only represent 10% of the total consumption. Williams (1973) and Vaccaro & Jannasch (1967) also found that only a monospecific bacterial population behaves according to the kinetics of the enzymatic model as proposed by Wright & Hobbie (1965, 1966).

As dark C-CO_2 consumption depends on different organic substrates, this rate can therefore be considered as an index for the estimation of total heterotrophic activity (Overbeck, 1979 a). Romanenko (1964) and

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Sorokin (1965) originally proposed the method for estimating the heterotrophic bacterial biomass, assuming that these organisms use 6% of external CO_2 for cellular biosynthesis. The miscalculations of heterotrophic bacterial biomass by the external C-CO_2 incorporation are due to the fact that this percentage is not constant and that not all bacterial groups have the same C-CO_2 requirements. Also, algal dark C-CO_2 fixation and intracellular refixation of respired CO_2 (Overbeck & Daley, 1973) cause errors.

The aim of this work is to estimate and compare both bacterial heterotrophic activity and phytoplankton autotrophic activity in the Embalse del Rio III Reservoir, because these are two basic processes of the carbon cycle in aquatic systems.

Study area

The Embalse del Río III Reservoir is a dam lake built for hydroelectric purposes in the Calamuchita Valley (Córdoba, Argentina) in 1936. The reservoir area is 45.3 km^2, the volume is 5.6×10^8 m^3, has a maximum depth of 46.5 meters and a mean depth of 12.2 meters. Its geographical position is: latitude 32° 11' S and longitude 64° 23' W, at 529.4 meters over the sea level. Annual precipitation is relatively low (760 mm in 1980) and there is little precipitation during July-September. Solar radiation on the lake becomes maximum in December (monthly average 5000 k cal $\cdot m^{-2} d^{-1}$) and minimum in June (monthly average 2150 k cal $\cdot m^{-2} d^{-1}$).

During summer periods there is an oxygen depletion in deeper layers. The most important phytoplankton characteristics are the occurrence of two algal blooms during the year: *Peridinium gatunense* at the end of summer to early autumn and Cyanophyta, Desmidiacea or Chlorococcales during end of winter and early spring (Boltovskoy *et al.*, 1980). The general characteristics of the reservoir are given in Table I.

MATERIALS AND METHODS

Samples were taken at a central station of the reservoir from March to September of 1981. They were collected with a Van Dorn sampler from 0, 1, 2.5, 5, 7.5 and 10 meters for the primary production measurements, and in addition to these depths, from 15 meters, and 1 meter above the bottom for the determination of C-CO_2 dark fixation. The heterotrophic potential for glucose was only estimated at 2.5, 7.5 and 1 meter above the bottom.

Dissolved oxygen concentration was measured by Winkler method (Alsterberg's modification) and the total inorganic carbon concentration was determined following Standard Methods APHA (1971).

Primary production was measured in situ with the ^{14}C-technique (Steemann Nielsen, 1952). Bottles were incubated twice in the daytime

TABLE I

Mean and maximum values of physical, chemical and biological variables.

	Mean annual value	Extreme values
Temperature °C	16.7	7.7 - 25.5
Dissolved oxygen mg l^{-1}	7.1	0.0 - 10.2
Conductivity umho cm^{-1}	140.0	86.0 - 193.0
$C\text{-}CO_3^{=}$ mg l^{-1}	0.11	0.0 - 1.5
$C\text{-}CO_3H^-$ mg l^{-1}	15.8	10.1 - 22.9
CO_2 mg l^{-1}	0.2	0.0 - 1.2
$P\text{-}PO_4^{3-}$ µg l^{-1}	7.7	0.0 - 93.0
$N\text{-}NO_3^-$ mg l^{-1}	0.08	0.0 - 0.48
$N\text{-}NH_4^+$ mg l^{-1}	0.24	0.0 - 0.47
$Si\text{-}SiO_2$ mg l^{-1}	6.2	0.3 - 14.7
Chlorophyll a mg m^{-3}	11.7	0.0 - 96.0
POC mg m^{-3}	965.0	350.0 - 2717.0
PON mg m^{-3}	132.0	29.5 - 458.0

from sunrise to noon and from noon to sunset. After exposure water samples were immediately filtered through Millipore filters of 0.45 µm pore size. After fuming over concentrated HCl for 1 minute, each sample on the filter was placed in a scintillation vial with Bray's fluor. The ^{14}C radioactivity of the sample was measured in a liquid scintillation spectrometer. Anaplerotic $C\text{-}CO_2$ consumption was estimated according to Overbeck (1981).

Kinetic parameters were obtained applying equation:

$$\frac{C\mu t}{c} = \frac{K_t + S_n}{V_t} = T_t$$

solved by linear regression. We used four glucose concentrations and one blank (Table 2) prepared with D-(U-^{14}C-glucose) from Radiochemical Centre, Amersham, England, with a specific activity of 279 m Ci/m mol, and with non labelled glucose. The bottles were covered with aluminium foil and incubated in a bath with continous circulation of lake water for 1-2 hours. After incubation, the samples were fixed with acetic Lugol's solution and filtered through membrane filters of 0.2 µm pore size. Blanks of each series were immediately fixed after the addition of labelled substrate. The filters were put in vials with 10 ml of scintillation solution. The blank activity was substracted from the activity obtained in the other concentrations and these data were mathematically treated to obtain the respective kinetic parameters. We worked with a probability error of less than 0.05% ($P < 0.05$).

TABLE II

Experimental glucose solutions.

Concentration	^{14}C-Glucose/50 ml		Glucose non-labell./50 ml	Total glucose/l
	μCi	μg	μg	μg
Blank	0.308	0.21	–	4.20
1	0.308	0.21	–	4.20
2	0.308	0.21	0.92	22.60
3	0.308	0.21	1.84	41.00
4	0.308	0.21	3.69	78.00

RESULTS AND DISCUSSION

Dissolved oxygen reached high concentrations in the whole water column in winter. There was variability with depth with lower but still biologically significant values near the bottom in March, April and May (Table 3).

A remarkable increse in numbers of the dinoflagellate *Peridinium gatunense* was detected in March. Primary production rates were high (338.7 mg C m^{-2} d^{-1}) up to 2.5 meters depth and decreased abruptly as is common in eutrophic waters bodies with a high phytoplankton density. The highest value of heterotrophic glucose consumption (833 μg C l^{-1} h^{-1}) was also detected during this month at 2.5 meters depth. Contrasting with Overbeck's (1979 b) findings the photosynthetic rates and C-glucose consumption showed similar vertical patterns in the euphotic zone during March, April and May.

Anaplerotic C-CO_2 use as a rough measure of heterotrophic activity, was low at the surface but increased markedly at the bottom. The ratio between carbon consumed as glucose and carbon consumed in the anaplerotic manner (C-Glu/C-CO_2, Table 4) was 28.1 in the euphotic zone and 14.0 in the whole water column, suggesting the existence of a community with maximum potential for glucose in the euphotic zone in which phytoplankton density and activity were higher. These rates also suggest the existence of a community with less potential for this carbohydrate near the bottom.

The relationship between autotrophic and heterotrophic processes is seen in the ratios of glucose uptake velocity and of C-CO_2 consumption with primary production (C-Glu/PP and C-CO_2/PP). Through the former relationship, one can deduce that a maximum of 6.4% of organic matter produced was consumed as glucose in March. This percentage increased to 8.6 and 11.6 in May and June respectively. Overbeck (1979 b) found that

TABLE III

Experimental results: C-CO_2 and C-Glucose in μg C l^{-1} h^{-1} ; primary production (PP) in mg C l^{-1} h^{-1} and dissolved oxygen concentrations (DO) in mg l^{-1}

Depth	Date	III-1981			IV-1981				V-1981				VI-1981			
(m)	C-CO_2	G-Glu	PP	DO	C-CO_2	C-Glu	PP	DO	C-CO_2	C-Glu	PP	DO	C-CO_2	C-Glu	PP	DO
surface	4.3	–	12.6	7.2	20.9	–		6.7	190.3	–	2.1	8.8	15.8	–	0.89	10.5
1	13.1	–	4.5	7.3	167.9	–	–	6.8	14.7	–	1.5	8.8	35.8	–	0.87	10.5
2.5	16.1	833	2.4	6.8	10.5	210	–	6.7	18.1	122	0.5	8.8	17.3	238	0.28	10.5
5	14.0	–	0.8	5.6	10.8	–	–	6.7	17.8	–	0.1	8.0	9.3	–	0.04	10.5
7.5	38.2	300	0.6	5.6	4.8	121	–	6.7	6.6	105	0.1	7.3	34.8	34	–	9.7
10	26.6	–	–	5.5	4.5	–	–	6.7	5.3	–	–	7.2	19.6	–	–	9.7
15	6.1	–	–	5.5	4.6	–	–	6.4	11.8	–	–	7.2	4.1	–	–	9.7
bottom	103.9	363	–	4.5	0.7	156	–	5.8	12.1	88	–	6.9	28.8	120	–	9.3

TABLE III

Experimental results: C-CO_2 and C-Glucose in μg C l^{-1} h^{-1}; primary production (PP) in mg C l^{-1} h^{-1} and dissolved oxygen concentrations (DO) in mg l^{-1}

Depth	Date	VII-81			VIII-81				IX-81			
(m)	C-CO_2	C-Glu	PP	DO	C-CO_2	C-Glu	PP	DO	C-CO_2	C-Glu	PP	DO
surface	42.1	–	4.3	9.9	106.9	–	4.2	8.4	164.5	–	14.7	8.9
1	24.5	–	3.1	9.8	90.1	–	4.3	8.4	208.0	–	7.7	8.8
2.5	47.9	27	1.4	9.9	96.9	15	1.0	8.4	163.9	60	0.4	8.6
5	36.5	–	0.2	9.8	83.8	–	0.05	8.1	10.31	–	0.08	8.7
7.5	41.5	34	0.26	9.8	85.5	23	0.06	8.1	74.8	105	0.04	8.6
10	76.9	–	–	9.6	88.8	–	–	8.1	55.8	–	–	8.7
15	40.5	–	–	9.5	147.1	–	–	6.7	118.3	–	–	8.1
bottom	94.3	11	–	8.5	158.1	82	–	4.8	121.3	29	–	–

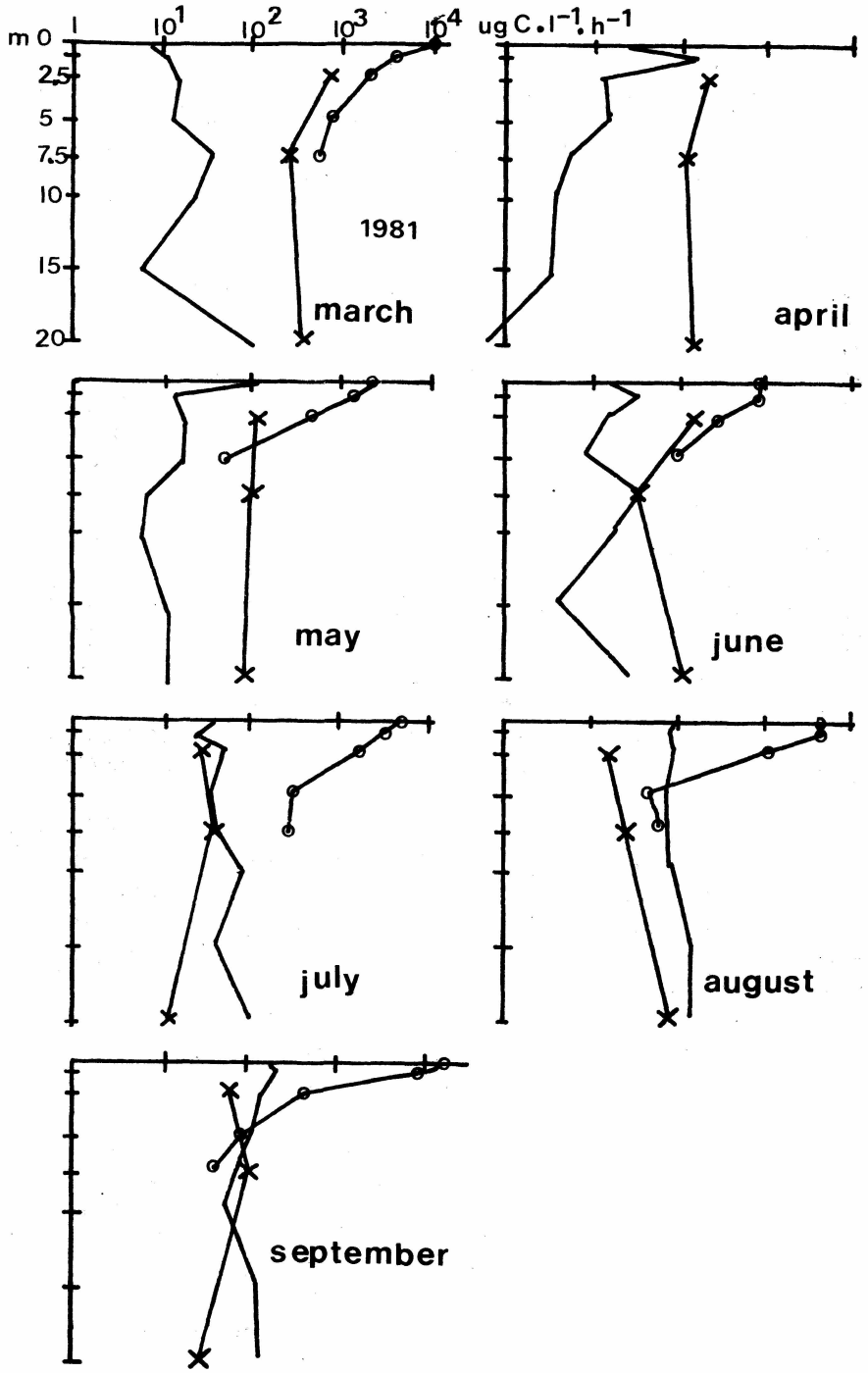

Fig. 1.– Vertical variations of primary production rates (o——o); C-CO_2 anaplerotic uptake (——) and C-glucose uptake (X ——X).

TABLE IV

Relationships between primary production rates (PP), C-CO_2 anaplerotic uptake and C-glucose uptake, integrated till 7.5 meters depth (o) or in the whole water column (x).

	III-81	IV-81	V-81	VI-81	VII-81	VIII-81	IX-81
PP mg C m^{-2} d^{-1} (o)	338.7	–	68.4	31.4	127.7	143.1	257.7
C-Glu μg C m^{-2} d^{-1} (o)	181.1	32.5	21.0	13.5	5.3	3.3	13.6
C-CO_2 μg C m^{-2} d^{-1} (o)	4.2	6.9	5.6	4.5	8.9	20.4	29.8
C-Glu μg C m^{-2} d^{-1} (x)	217.7	74.2	50.2	36.7	12.1	14.2	33.9
C-CO_2 μg C m^{-2} d^{-1} (x)	15.5	8.3	8.5	10.2	29.0	60.2	60.2
C-Glu/C-CO^2 (o) %	28.1	4.6	3.8	3.0	0.6	0.1	0.4
C-Glu/C-CO_2 (x) %	14.0	8.9	5.9	3.6	0.4	0.2	0.5
C-Glu/PP (x) %	6.4	–	8.6	11.6	0.9	0.9	1.2
C-CO_2/PP (x) %	0.4	–	1.4	3.2	2.2	4.2	2.1

this value was 10% for PluBsee; 3.3% for Kellersee; 1.9% for Gr.Plönersee and 1.7% for Schönsee.

During April the heterotrophic activity estimated by heterotrophic potential for glucose was 74.2 μg C m^{-2} d^{-1} and 8.3 μg C m^{-2} d^{-1} by dark CO_2 consumption. Vertical variation of heterotrophic activity estimated for CO_2 showed a high value (167.9 μg C l^{-1} h^{-1}) at one meter depth. This figure was similar to the one observed for glucose consumption at lowest depths. But at the deepest sample in April, the C-CO_2 consumption dropped to 0.7 μg C l^{-1} h^{-1}.

Primary production and glucose consumption were less in May and June while anaplerotic C-CO_2 use maintained its level. This suggest that the bacterial community may be using other substrates as a source of carbon. In June the anaplerotic consumption of C-CO_2 equalled that of glucose at 7.5 meters depth. There was a low phytoplankton density and a dominance of *Melosira granulata* at this time.

A slight increase in primary production values was seen in July, August and September. Heterotrophic activity estimated by glucose consumption dropped markedly, while C-CO_2 increased greatly, being higher than C-glucose at all depths. The C-glucose/C-CO_2 ratio indicates that the heterotrophic activity moves toward the uptake of glucose or to another organic substrate, and its values were lower than 1 during this three months. Dark C-CO_2 fixation reached 2 and 4% of that produced by phytoplankton while glucose did not represent 1% of photosynthetisized carbon. Overbeck (1979 b) found that dark CO_2 fixation was around 3.6% of light fixation.

It can be assumed, from the dissolved oxygen concentrations obtained through the study, that the dark C-CO_2 consumption may be due to heterotrophic bacteria, although the possibility of consumption by chemoautotrophic bacteria can't be excluded.

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