

## HORIZONTAL DISTRIBUTION OF PHOTOTROPHIC BACTERIAL POPULATION IN AN IRREGULARLY-SHAPED MEROMICTIC BASIN OF BANYOLES LAKE (BANYOLES, SPAIN)

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### RESUM

S'ha estudiat la distribució de diferents paràmetres relacionats amb el desenvolupament de bacteris fototròfics del sofre a sis punts de la mateixa fondària de C-IV, una cubeta meromíctica de l'estany de Banyoles. S'han detectat dos grups de paràmetres: un de directament relacionat amb la proximitat del sediment, i l'altre (Bacterioclороfil·la *e* i *a*, sulfhídric i ferro total), amb la presència de dues *subcubetes* (enfonsaments) dins de la mateixa cubeta. En aquest article es proposen explicacions biològiques i físiques per tal d'entendre les diferències existents. La proximitat al sediment i l'estabilitat de la meromixi són els factors més probables que afecten la distribució horitzontal de les poblacions de bacteris fototròfics a la cubeta IV.

### RESUMEN

Se ha estudiado la distribución de distintos parámetros relacionados con el desarrollo de bacterias fototróficas del azufre, en seis puntos de igual profundidad de C-IV, una cubeta meromíctica del lago de Bañolas. Se han detectado dos grupos de parámetros: Uno directamente relacionado con la proximidad al sedimento y otro (Bacterioclороfila *a*, *e*, sulfhídrico y concentración de hierro total) con la presencia de dos subcubetas (hundimientos) dentro de la misma cubeta. En este artículo se proponen explicaciones biológicas y físicas que justifican las diferencias halladas. La proximidad al sedimento, así como la estabilidad de la meromixis, son factores probables que afectan a la distribución horizontal de las poblaciones de bacterias fototróficas en la cubeta IV.

### ABSTRACT

Six points at the same depth were checked in C-IV, a meromictic basin in Banyoles lake, for several parameters dealing with the development of sulphur phototrophic bacterial populations. Two main groups of parameters have been detected: one directly related with the proximity to the sediment and another (Bchlор *e* and *a*, sulphide and total Iron) agrees well with the presence of two «holes» inside the basin. In this paper physical and biological explanations are given in order to understand the differences found. Proximity to sediment and meromixis stability appear as the most probable factors affecting horizontal distribution of phototrophic bacterial population in C-IV.

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**Key words:** Banyoles lake, horizontal distribution, patchiness, meromixis, physical Limnology.

## INTRODUCTION

Few works have been made dealing with horizontal distribution of both physico-chemical and biological parameters. However, it is known that horizontal differences in such distributions are closely related to summer estratification conditions (Wetzel, 1975; Margalef, 1983). Some parameters as oxygen distribute horizontally depending on related biological activities such as either algae production or metabolic consumption (Buscemi, 1958).

On the other hand the complex morphometry of the lakes promotes large horizontal variations (Wetzel, 1966) since they determine particular conditions.

This study is centered in the chemocline of C-IV a meromictic basin of Banyoles lake. It has a very irregular shaped bathimetry (Moreno-Amich & Garcia-Berthou, 1987) and the chemocline is located at 13 meters during August.

Limnological sampling in lakes is usually done at one sole point, at the maximal depth of the basin, when such lake is not too large. That sampling provides a global idea about the physico-chemical and biological status of water column. In C-IV, where phototrophic bacteria population are studied, the presence of light and sulphide is a determining factor for their development.

On the other hand, the presence of soluble ferrous iron (Fe II), transforms sulfide in a limiting factor (concentrations below 10  $\mu\text{M}$ , unpublished data). During the work period of time (July 1988) the chemocline in C-IV was located at 13 meters. Horizontal distribution of *Chlorobium phaeobacteroides* population, all over this chemocline can be quite different in relation to the proximity of sediment.

Each point can be defined by different physico-chemical conditions, leading to a diverse biological response of population. The aim of this work is to elaborate a distribution map of *Chlorobium phaeobacteroides*, on the plane defined by the chemocline.

## MATERIAL AND METHODS

### Study area

C-IV is a meromictic basin located in the north of Banyoles lake. It is irregularly shaped, containing three sub-basins of 16, 18 and 19 meters depth respectively. Two of these «holes» have an area with bottom incoming water. Banyoles lake is located near the town of Girona and belongs to a complex karstic system. An arbitrary coordinates system was used in order to have two-dimensional data of the surface located at 13 meters. Y-axis corresponds to South-North line. Points in the plane at 13 meters depth are given under the form (a,b) where a is the abscise and b the ordinate. The chosen depth corresponds to the redoxcline, and it is the place where the bacterial plate was found (fig. 1).

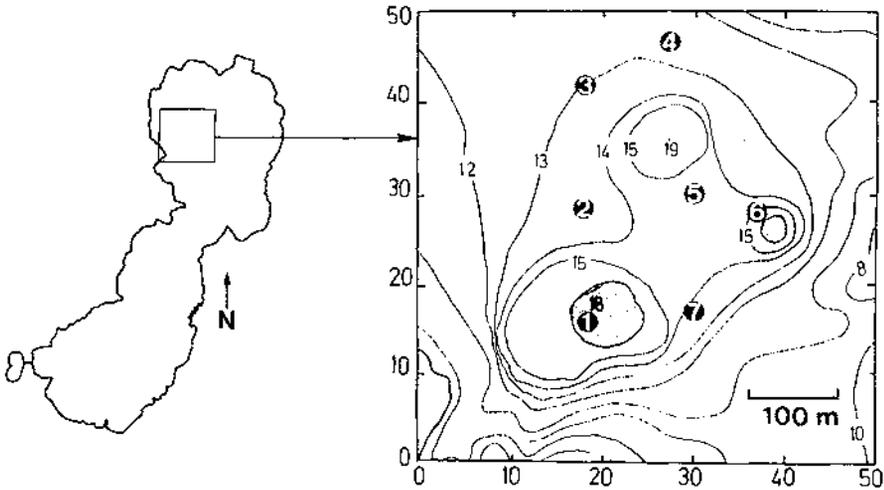


Figure 1. Location and bathymetry of C-IV, the studied basin, in Banyoles lake. Notice its irregular shape as well as the two «holes» inside.

### Field measurements

Before sampling, vertical profiles of physico-chemical parameters were performed in order to locate the bacterial plate. Temperature was measured with a Crison T-637 thermistor. Conductivity was taken with a YSI (Yellow Spring Instruments) model 33. Duplicate water samples were taken at different points in the same depth (13 meters) with a pumping system and were stored in hermetically closed bottles.

### Chemical analyses

pH was measured with an Orion glass electrode (Orion 81-85). Total iron concentration was calculated using the thiocyanate method (Vogel, 1978). Ammonia concentration was tested with ammonia selective electrode (Orion 95-12). Samples for sulphide analyses were stored in screw capped tubes and preserve to the oxidation by addition of 10 ml of SAOB (antioxidant buffer). Analyses were performed by potentiometric methods with an Orion (94-16) sulphide selective electrode and Orion (90-92) double junction reference electrode.

Pigment were analyzed by filtering 500 ml of field samples through membrane filters (Sartorius; 0.45  $\mu\text{m}$  pore diameter) previously covered with a thin layer of  $\text{MgCO}_3$  (Montesinos 1982, Takahashi & Ichimura 1968).  $\text{MgCO}_3$  layer containing cells was suspended in 3 ml of 90 % acetone. Afterwards, the extract was centrifugated and the clear supernatant was used for spectrophotometrical analyses.

## RESULTS AND DISCUSSION

Investigations of horizontal distribution of determined parameters are rarely performed. However, they are needed, because these differences cannot be predicted at present.

In this work several physico-chemical parameters were measured trying to define which parameters distribute horizontally together with population of phototrophic bacterial populations.

The accuracy of sampling was a fundamental issue, since working on the chemocline (a gradient) implies that little errors in the vertical measurement could significate a considerable change in the value of the parameter since it ranges from zero to its maximum value in a few centimeters. In this work, conductivity and water samples were taken separately and, nevertheless, the maximum of conductivity, located at coordinate point (3,3) coincides with other peaks of other parameters measured from the water samples taken independently. Observing high values in relation to low ones a sampling error could be deduced. Thus, if values increase with depth one can think that sample was taken at a few centimeters deeper. Nevertheless, pH distribution diagram is a good control that eliminates this possibility. High values of pH coincide with maxima. In the hypothetic case that samples were from a slightly deeper point, pH values would range down, (Fig. 2) and this is not the case.

Table 1 shows two points (1 and 4) having higher values than the rest. Almost all parameters have a maximum at this point. Figure 3 compiles a group of three parameters (ammonium, pH, conductivity and Bchl *a*) having a similar pattern of distribution. This pattern differs clearly from that for the other parameters (sulfide, total iron, and bacteriochlorophylls *a* and *e* Fig. 3).

The pattern of distribution in fig 2 indicates that there exist higher values of pH, conductivity and ammonia in the coordinate (3,3) approximately, and can be compared with other biological parameters represented in figure 3. In this case there are two peaks at coordinate (3.3,3) and (1.9,1.5). It has to be noticed the coincidence of those two peaks with the presence of two «holes» of 18 and 19 meters depth respectively. Some physical considerations on this topic are discussed below.

Water flux enterig C-IV the same day of sampling can be estimated using the methods described by Roget & Casamitjana (1987) and is about  $7030 \text{ cm}^3 \cdot \text{sec}^{-1}$ . This water has a greater conductivity and therefore is denser than the remainder. The density differences between water of monimolimnion (point no 1 at 14 meters depth) and that for monimolimnion (same point at 11 meters depth) can be estimated in  $6 \times 10^{-4} \text{ g} \cdot \text{cm}^{-3}$ . Therefore, freatic water tends to accumulate on the bottom forming a boundary layer. Thus, the energy generated by the wind induces turbulent processes leading to the mixing of monimolimnion waters into mixolimnetic ones.

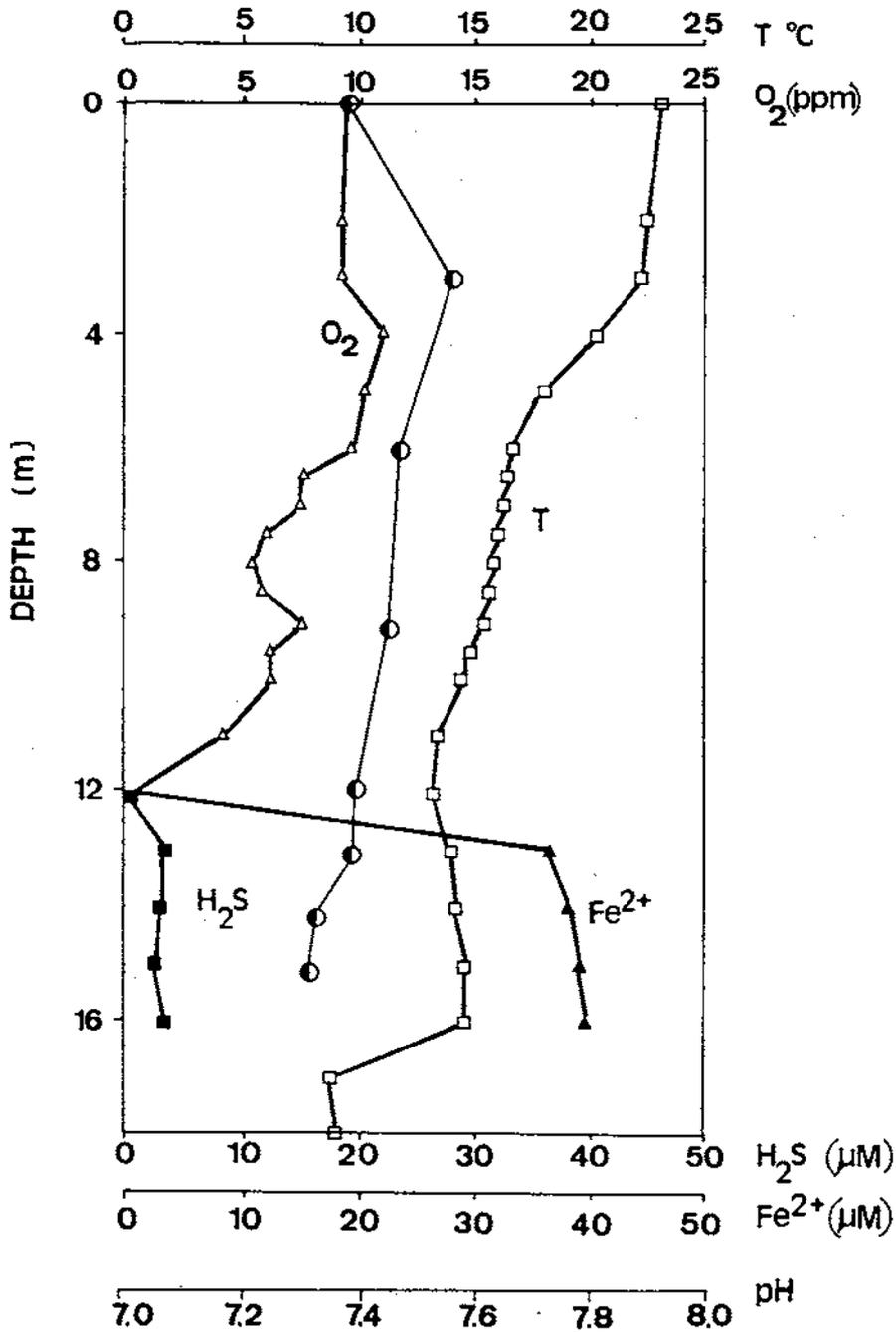


Figure 2. Vertical profile of measured parameters, in C-IV, the same day of the study.

Table 1. Numerical values obtained at the different points checked.

Point	X-axis	Y-axis	Conduct.	pH	Ammnonia	Sulphur	Total Iron	Bchlor e
1	1.8	1.5	1996 ± 4	6.84	56	0.30	35.3 ± 0.5	78.94 ± 4.6
2	1.75	2.85	1922 ± 38	7.03	54	0.10	5.70 ± 1.4	26.561
3	1.76	4.2	1911	7.12	63	0.19	13.35 ± 3.6	45.104 ± 3
4	2.7	4.65	1360					
5	2.95	3	1982 ± 8	7.02	60	0.18	24.24 ± 2.8	76.745 ± 2
6	3.7	2.8	1915 ± 55	7.09	61	0.10	5.42 ± 1	26.25 ± 2.3
7	3.0	1.7	1584 ± 104	7.09	56	0.04	3.3 ± 0.74	16.40 ± 4.5

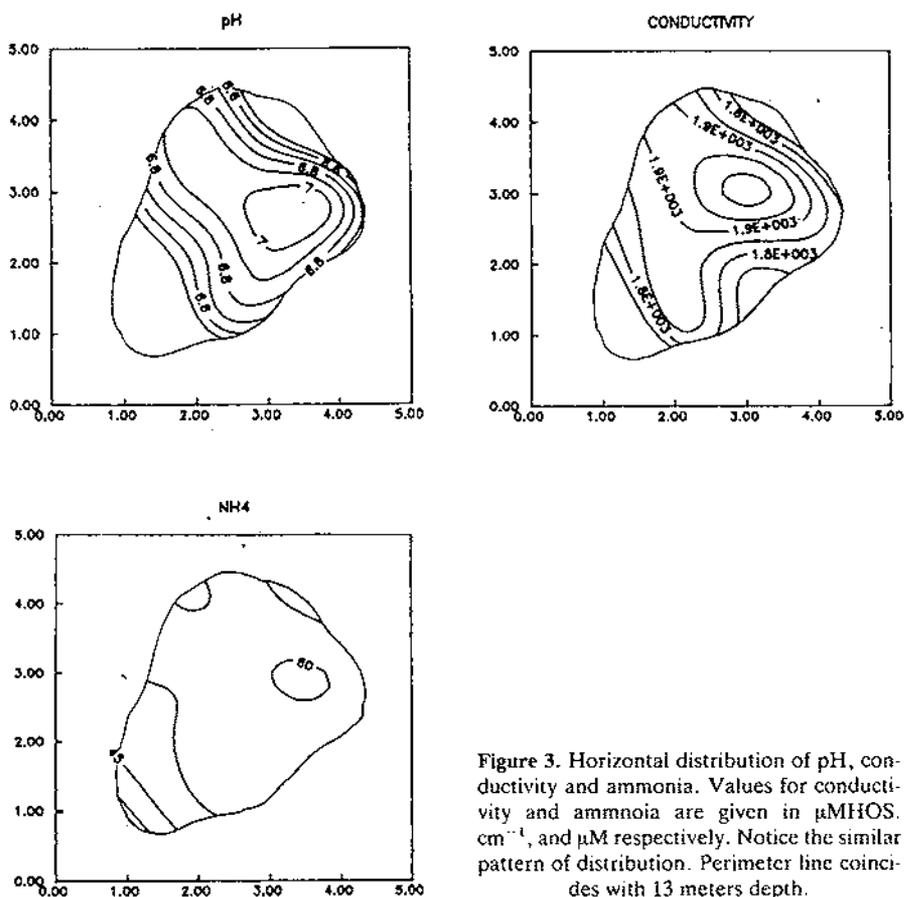


Figure 3. Horizontal distribution of pH, conductivity and ammonia. Values for conductivity and ammonia are given in  $\mu\text{MHOS. cm}^{-1}$ , and  $\mu\text{M}$  respectively. Notice the similar pattern of distribution. Perimeter line coincides with 13 meters depth.

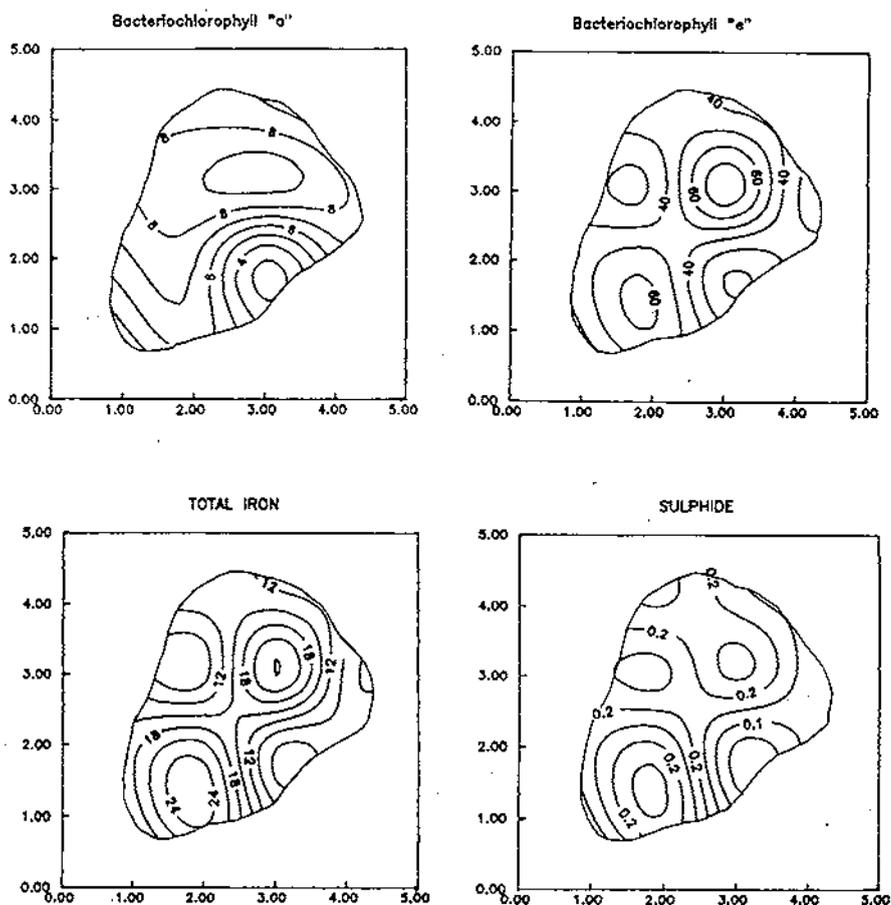


Figure 4. Horizontal distribution of Bacteriochlorophylls *a* and *e* in  $\mu\text{g.L}^{-1}$ , total iron ( $\mu\text{M}$ ) and sulphide ( $\text{mM}$ ). Notice the similar pattern of distribution, with maxima coinciding with the two holes.

The turbulent energy generated by the wind decreases with depth, but specially with horizontal area and at 14-15 meters depth of points 1 and 4, this energy is very low. This feature confers to this zone a great stability and make the conductivity to be higher. As a consequence of this effect, the concentration of chemical and biological parameters are also higher. Sulphide, for example, is produced in compact sediment (i.e. not suspended) and it is horizontally homogeneized by diffusion throughout the basin. Sulphide concentration depends fundamentally on both the distance to sediment (the higher the proximity the higher the concentration) and the proximity to an area of high stability.

Since sampling point number 3 is more close to sediment than point 2, at 13 meters depth, its sulphide concentration is higher than the point number 2 at the same depth. On the other hand sulphide concentration of point 1 is higher than point 3 since it is located in a higher stability area than point 3 and the wind-generated mixing energy is lower.

In table 2, statistical correlations between bacteriochlorophyll *e* and the other parameters tested are shown. A high correlation factor with Fe(II) was found. This relationship was also found in C-IV during 1987 (unpublished data), but in this case at different time and depths. This feature can be understood in a close physical relationship between Chlorobiaceae and Fe(II) is assumed.

**Table 2.** Statistical correlation between bacteriochlorophyll *e* and differents parameters measured.

Conduct.	pH	Ammnonia	Sulphur	Total	Iron
0.7267	0.4877	0.0223	0.7775	0.9311	

There exists a common maximum for all measured parameters in the surroundings of point 5. This point is located on a wide sediment area one meter below (14 meters depth). The activity of this sediment appears to be responsible for higher H<sub>2</sub>S values in the chemocline. On the other hand the presence of a 19 meters depth hole nearby this sediment area gives the physical stability to make the physico-chemical conditions persistent. This fact can be monitored by the conductivity. Therefore, the phototrophic bacterial growth at this point would be higher than in the rest of chemocline. Eddy diffusional processes tend to spread and homogenate horizontally the *Chorobium phaeobacteroides* population. The physical stability given by the «holes» results in an accumulation in these areas since they are mixing processes acting on these points are less important.

A dynamic process could also influenciate in the horizontal distribution found. It is the oscillating behaviour of meromixis in C-IV. Chemocline situation ranges up from 16 meters depth in early spring to 12 meters depth during late summer (Garcia-Gil et al., 1987). Therefore, when phototrophic bacteria population begins to grow, it remains accumulated inside the holes (Fig. 1), from 16 meters depth to the bottom. Chemocline reaches upper positions, over time course, and the area that determines is higher. When meromixis reaches its maximum stability the chemocline area neighbouring a hole has a higher bacterial concentration and produces the patchiness found, since the time of growth has been much higher in those points.

As can be deduced, there are a lot of parameters affecting spatial-temporal distribution of phototrophic bacteria in natural environments. The horizontal distribution of these populations shows that their development is determined by the own characteristics of the lake where they are placed.

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