

# BIOLOGICAL STUDIES OF SPANISH MEROMICTIC AND STRATIFIED KARSTIC LAKES

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## ABSTRACT

Surveys have been carried out in over 40 lakes in the Pre-Pyrenees, Cuenca mountains and Banyoles karstic areas in Spain. About 18 of these lakes are deep enough to become stratified and have been studied in more detail. Here we discuss the vertical distribution and species composition of their planktonic communities, and their relationship to physico-chemical parameters. Meromictic and stratified karstic lakes are characterized by the large accumulations of organisms at the redoxcline. Sharp gradients of oxygen and reduced compounds are found in this region as a consequence of the activities of the organisms. Different organisms form abundance maxima at their preferred depths further contributing to the stratification. Special attention is given to these communities at the oxic-anoxic interface. Usually, rotifers accumulate in the microaerophilic zone. Algae may be found at the zero oxygen depth. Phototrophic anaerobic bacteria are found underneath when enough light reaches the interface. Different communities of ciliates appear in the aerobic waters, in the oxycline and in the anaerobic waters respectively.

## INTRODUCTION

Karstic lakes originate by limestone and/or gypsum dissolution. They have a peculiar morphometry, characterized by circular sinks with steep walls and a high relative depth (the ratio of maximum depth to mean diameter, see table 1). These features, together with a high mineralization of the bottom waters due to their dissolution origins, favor the stratification of the water layers. Permanent meromixis, however, is a peculiar condition found only in a few lakes. Many karstic lakes with similar morphometry and substratum are not meromictic, such as those next to the locality of Cañada del Hoyo (Cuenca), where only Laguna de la Cruz is permanently meromictic, out of a total of seven dissolution lakes. Considering the whole karstic area in the mountains of Cuenca, where we have studied more than 35 dissolution lakes and small water bodies, only two (Laguna de la Cruz and Lake El Tobar) were found to be meromictic. Several of these lakes can be seen in Plate I. Laguna de la Cruz presents a biogenic meromixis, due to enrichment of the monimolimnetic water in calcium, magnesium and iron bicarbonates. Lake El Tobar shows a crenogenic meromixis

where the monimolimnion is constituted mostly by a sodium chloride brine (table 1). Another karstic area exists around Lake Banyoles (Girona). The main lake itself is a polje constituted by six main basins, but only one of them is permanently meromictic. There are about 15 small lakes surrounding the main lake and only one of them is meromictic (Lake Vilar). Thus, we find a continuous spectrum of lakes with different degrees and schedules of stratification. The most extreme and rare case being permanent meromixis.

One of the most striking features of all these lakes is the sharp stratification of organisms, which is determined by their own activity. This is common to both meromictic lakes and to those stratified during an important part of the year. Actually, these stratified lakes were first studied for their accumulation of sulfur photosynthetic bacteria at the oxic-anoxic boundary (ABELLÀ *et al.*, 1980, 1981, 1985; GASOL *et al.*, 1990; GUERRERO & ABELLA, 1978; GUERRERO *et al.*, 1978, 1980, 1985, 1987; MAS *et al.*, 1990; PEDRÓS-ALIÓ *et al.*, 1986; VICENTE & MIRACLE, 1984, 1988, 1991). Additional information can be found in GUERRERO & PEDRÓS-ALIÓ (1992). More general limnological studies were only carried out later on.

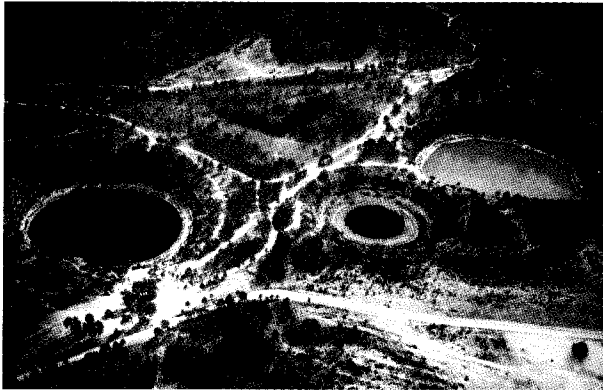


Plate 1. Aerial photograph of lakes La Cruz, Lagumillo del Tejo and El Tejo.

This paper will summarize biological studies of the massive accumulations of organisms at such boundary. We hope to demonstrate that such organisms form a rather distinct but widespread community. The paper will focus on the meromictic and stratified karstic lakes in the Cuenca mountains, the Banyoles region, and the Pre-Pyrenean mountains in Huesca and Lleida. Similar accumulations of organisms at interfaces can be found in coastal stratified lagoons such as La Massona, Girona (RIERA & ABELLÀ, 1986, 1991; GUERRERO *et al.*, 1987; DOMINGUEZ-PLANELLA *et al.*, 1987), Cullera, Valencia (MIRACLE & VICENTE, 1983, 1985; ROJO & MIRACLE, 1989) or El Cibollar, Mallorca (MOYÀ *et al.*, 1987). Some information about these coastal lagoons can be found in table 1, but their detailed discussion is beyond the scope of the present review.

## MATERIAL AND METHODS

Since the studies summarized here have been carried out by many researchers in different years and ecosystems, the methods given are those we consider optimal after several years of experience in stratified lakes.

### Sampling

Two characteristics of the stratified lakes studied require a very careful sampling. First, the systems are sharply stratified and, second, the lower layers are always anaerobic and rich in reduced compounds such as sulfide. Thus, considerable care has to be given both to exact positioning of the sampling devices and to protect anoxic water samples from oxidation. The position of the boat can be fixed by exten-

ding two stretched steel wires perpendicular to each other and tightly fastened at their crossing point and fixed at the lake shores. The boat is then firmly tied to the wires. Several probes are lowered to determine vertical profiles of oxygen, temperature, conductivity, and light penetration. Since sulfide interferes with some oxygen electrodes, we recommend the use of membrane-covered amperometric silver-gold electrodes. The probes are lowered at a distance from each other to avoid interference among them and disturbance of the fine layering at the oxic-anoxic interface. The depths to be explored in more detail are determined on the basis of the profiles found.

Two different systems can be used for close-interval sampling (e.g. 5 cm). The first is an improved version of the fine-layer sampler described by JØRGENSEN *et al.*, (1979) and is shown in fig. 1A. This system consists of a conical inlet device, connected to a surface pump, which smoothly sucks the organisms towards the tubing, minimizing turbulence and escape responses of the larger organisms such as rotifers and crustacean nauplii and juveniles. Larger members of the zooplankton, such as cladocerans and adult copepods, are not properly sampled by this system. The double cone allows pumping large volumes of water from a given depth, with minimal disturbance of the stratification. The sampler is made of three Plexiglas cones: two hollow and one solid (fig. 1A). The lower cone is loaded with lead pellets as ballast. The solid cone fits on a specially carved rim of the lower cone and is glued to it. The upper hollow cone is attached to the lower one through three screws (only two appear in the figure) leaving a 1 cm circumferential aperture between them. The transition from the outer inlet to the small diameter hose is, thus, smoother than in the original design of JØRGENSEN *et al.*, (1979). A screwable connector allows attachment between the cone (by the screw) and the hose (through clamping of the hose to the upper portion of the screw). The hose and screw can be easily removed by unscrewing the cone. This is very convenient for transport or for changing hoses of different lengths for sampling lakes of different depths. Ideally, a peristaltic pump should be used on the boat. Submerged pumps next to the inlet should be avoided because vibrations of the pump may disrupt the fine layering. The device is sustained by a measuring tape. These tapes are non stretchable and, thus, provide the most precise and convenient way to determine the exact depth at which each sample is taken.

The second sampling system is a modification of the syringe systems devised by BAKER *et al.*, (1985) and MITCHELL & FUHRMAN (1989) and is shown in fig. 1B.

Table 1. Representative values of several physico-chemical parameters in the different layers of Spanish karstic lakes during stratification. Lakes are arranged according to their mixing regime and to their sulfate content. Next to each lake, relative depth (zr), maximal depth (zmax), and geographical coordinates are provided. Abbreviations for geographical areas are: B, Barcelona; CU, Cuenca; GI, Girona; HU, Huesca; L, Lleida; M, Mallorca; V, Valencia. Credit is given to appropriate references in the text and to C. Abellà and G. Moya personal communications.

Lake	Z <sub>r</sub> (Z <sub>max</sub> )	Conductivity mS cm <sup>-1</sup>	Eh mV	Alkalinity meq l <sup>-1</sup>	Sulfate mM	Sulfide mM	Phosphate μM	Nitrate μM	Ammonia μM
<b>1. Meromictic lakes</b>									
<b>1.1. Crenogenic</b>									
El Tobar (CU)	13 (19.5)	40°33'N/2°3'W							
Mixolimnion		0.6	+300	3.0	0.3	0	<0.1	14	8
Oxic-anoxic interface		20	+270	5.0	0.5	0	0.4	1	70
Monimolimnion		200 <sup>a</sup>	+25	30.0	11.7	0.3	20	0	1200
<b>1.2. Biogenic</b>									
<b>1.2.1. Low sulfate</b>									
Laguna de la Cruz (CU)	18 (24)	39°59'N/1°52'W							
Mixolimnion		0.5	+375	5.1	0.1	0	<0.1	0.5-1	30
Monimolimnion		1.5	+20	20.0	0.03	0.015	80	0	3750
Montcortés (L) <sup>b</sup>	2.8 (30)	42°20'N/1°0'E							
Mixolimnion		0.3	+200	2.7	1.0	0	<0.1	0-3	3
Monimolimnion		0.3	ND	3.8	2.0	0-0.2	<0.1	0	30
<b>1.2.2. High sulfate</b>									
Banyoles III (GI)	3 (30)	42°8'N/2°45'E							
Mixolimnion		1.2	+275	4.0	5.2	0.0	0-2	5-10	10
Monimolimnion		2.2	-110	5.0	9.5	1.5	0-7	0	100
Vilar (Banyoles, GI)	2.3 (9)	42°8'N/2°45'E							
Mixolimnion		1.1	+130	3.5	5.3	0	0.2	350	15
Monimolimnion		1.5	-175	8.8	11.6	5.9	7.0	0	700
<b>2. Holomictic lakes</b>									
<b>2.1. Low sulfate</b>									
Coromines (GI)	2.6 (5)	42°8'N/2°41'E							
Epilimnion		0.6	-80	5.0	0.7	0	6.0	500	7.5
Hypolimnion		0.8	-380	6.0	0.3	1.5	55.0	0	40-1800
Negre I (GI)	6.5 (5.2)	42°8'N/2°41'E							
Epilimnion		0.6	-100	5.7	0.4	0	3.5	20	4
Hypolimnion		0.8	-275	ND	0.4	0.2	15.3	0	15-100
Laguna del Tejo (CU)	16 (32)	39°59'N/1°52'W							
Epilimnion		0.47	+150	5.3	0.05	0	0.01	0.5-1	20
Hypolimnion		0.51	-25	6.5	0.04	0.002	0.2	0	48
Lagunillo del Tejo (CU)	11 (11)	39°59'N/1°52'W							
Epilimnion		0.52	+300	5.5	0.04	0	0.1	0.1	5
Hypolimnion		0.64	+60	6.0	0.02	0.004	0.2	0	100
La Parra (CU)	11 (16)	39°59'N/1°52'W							
Epilimnion		0.41	+300	4.3	0.08	0	0	0.5-1	0.6
Hypolimnion		0.32	+178	4.8	0.05	0	0.01	0	150

Table 1 (continuation)

Lake	$Z_r(Z_{max})$	Conductivity mS cm <sup>-1</sup>	Eh mV	Alkalinity meq l <sup>-1</sup>	Sulfate mM	Sulfide mM	Phosphate μM	Nitrate μM	Ammonia μM
Las Cardenillas (CU)	9 (12)	39°59'N/1°52'W							
Epilimnion		0.4	+320	4.3	0.10	0	0	0.5-1	0.4
Hypolimnion		0.3	+90	4.5	0.05	0	0.1	0	63
Lagunillo de las Cardenillas	10 (6)	39°59'N/1°52'W							
Epilimnion		1.28	+350	15.0	0.10	0	0.5-1	0.5-1	3
Hypolimnion		1.31	+350	15.0	0.10	0	0	0	1
<b>2.2. High sulfate</b>									
Cisó (Banyoles, GI)	9.2 (8)	42°8'N/2°45'E							
Epilimnion		2.0	+130	4.0	10	0	0-3.0	0-1.3	170
Hypolimnion		1.6	-310	7.6	7	2.0-7.5	0-3.0	0	800
Nou (Banyoles, GI)	6.8 (7)	42°8'N/2°45'E							
Epilimnion		1.8	+10	7.0	11.8	0	0-0.5	0.5	0.8
Hypolimnion		2.1	-225	11.5	12.5	1.2	1.0	<0.05	ND
Arcas-2 (CU)	28 (14.5)	39°59'N/2°8'W							
Epilimnion		2.4	+400	3.2	18	0	0.05	10-20	15
Hypolimnion		2.4	-100	4.5	17	0.5-2	.05-.5	0	350
Estanya (HU)	12.3 (20)	42°2'N/0°32'E							
Epilimnion		1.9	+360	2.3	10	0	0-2.0	8.5	90
Hypolimnion		1.5	-35	3.0	8	0.5	0-0.4	2.0	ND
<b>3. Coastal lagoons</b>									
Massona (GI)	1 (10)	42°13'N/3°8'E							
Mixolimnion		52	+200	4.5	5.0	0	0-2	1-100	0.1-2
Monimolimnion		35	-400	5.5	40.8	0.6	10-100	0	ND
Remolar (B)	0.3 (4)	41°17'N/2°4'E							
Mixolimnion		4-20	+40	ND	1-27	0	0.5	ND	ND
Monimolimnion		35	-330	ND	7-42	3.1	10-40	ND	ND
Cullera (V) <sup>c</sup>	0.5 (7)	39°8'N/0°14'W							
Mixolimnion		1.1	+300	3-5	4.4	0	0	20-200	25
Monimolimnion		40	-150	6-8	16.5	4.0	20.6	0	6000
Cibollar (M)	8.2(3.7)	39°48'N/3°6'E							
Mixolimnion		10	ND	4.8	6	0	0.5	15	ND
Monimolimnion		41	ND	6.1	25	2.5	35	8	ND

<sup>a</sup> The high conductivity is due to a sodium chloride brine. Typical chloride concentrations are 0.1, 500 and 6800 mM at the mixolimnion, interface and monimolimnion respectively.

<sup>b</sup> Meromictic in 1975-76, but mixed in 1979.

<sup>c</sup> Meromictic before a flood in 1982.

The syringes are filled simultaneously at the desired depth by the action of a vacuum pump connected to the device. A detailed instantaneous image of the vertical distribution of the organisms can be obtained quickly and avoiding problems created

by surface waves. The model shown in fig. 1B has 33 syringes in a one meter copper column, but other lengths and spacing among syringes can be used as desired. This systems works only down to approximately 2 m before hydrostatic pressure

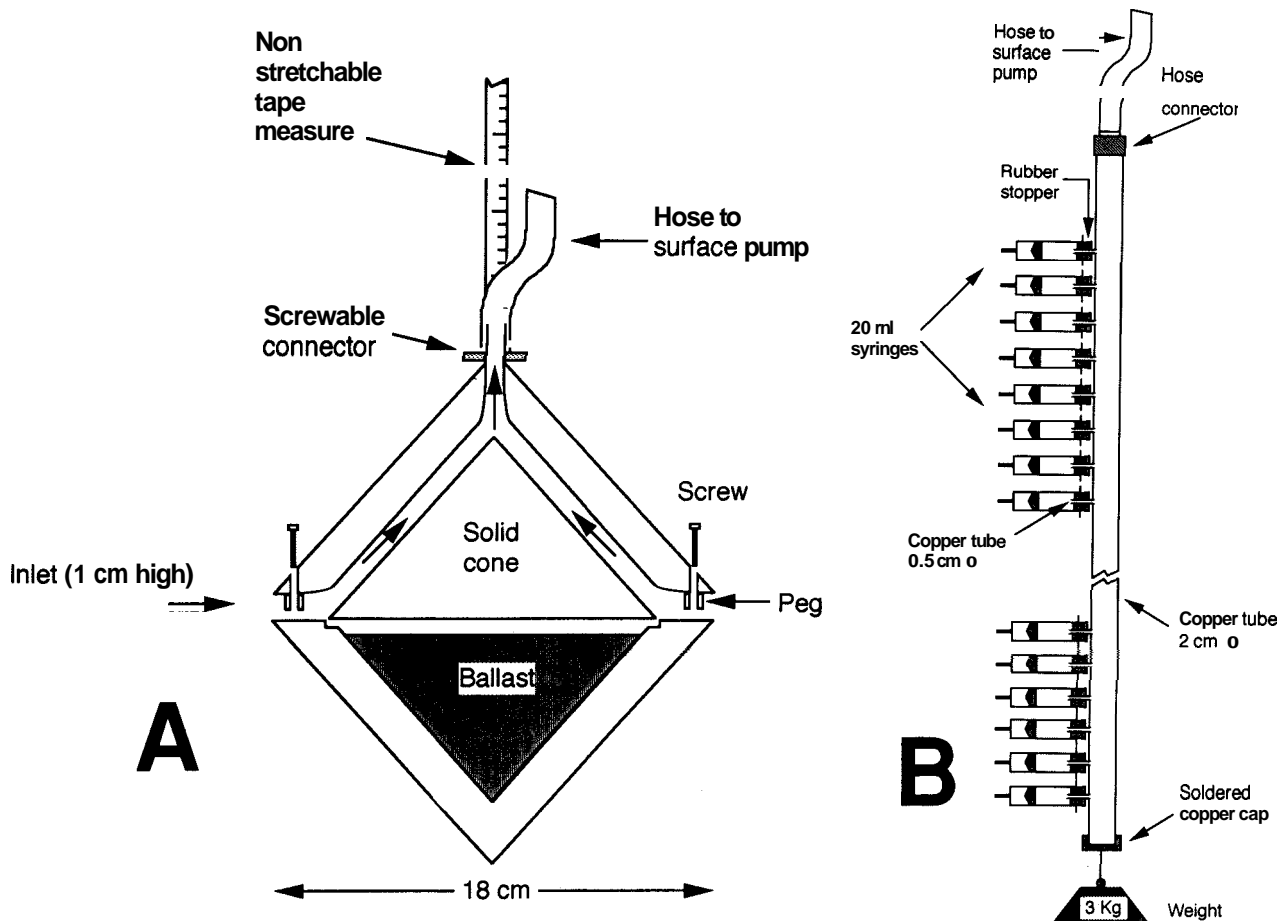


Figure 1. Devices for sampling sharply stratified water columns. A) Modified double cone. B) Syringe system

fills the syringes spontaneously (at 3 to 4 m). For such depths, air can be pumped into the system from the surface until it reaches the desired depth. This may work, depending on the syringes used, down to 10 or 15 m. For better performance at depth, however, a messenger triggered system such as that of BJØRNSSEN & NIELSEN (1991) is recommended.

The systems are lowered to the desired depth with utmost care not to disturb the fine stratification of organisms. A good procedure consists of slowly lowering the device to the desired depth, then very slowly and smoothly moving it sideways (syringes-side first in the case of the syringe system) to sample an undisturbed section of the water column.

In order to determine the exact depth of the peaks of phototrophic organisms samples are taken at 5 cm intervals and filtered immediately through Whatman GF/C glass fiber filters. The color of the filters shows which is the dominant organism at each depth, and where the layers of algae and phototrophic bacteria start and end. Samples are then collec-

ted at the appropriate depths taking into account that accumulations of zooplankton are usually found a few cm above the layers of algae and bacteria.

Water from the pump can be diverted into a flow-through chamber with electrodes for instantaneous determination of oxygen, pH, and Eh (fig. 2). In all cases, samples have to be protected from direct sunlight and warm surface temperatures. Since the bottom waters are generally anaerobic, samples must be distributed into appropriate bottles, tightly capped and impermeable to oxygen, and fixed immediately upon reaching the surface. When samples have to be fixed for counts of organisms, the fixative can be previously dispensed into appropriate bottles and these are carefully filled (to avoid turbulence) directly from the pump outlet. Care has to be taken to avoid spilling fixative. In the case of samples for reactive compounds such as oxygen or sulfide, and those which have to be incubated, the bottles are flushed with three times the bottle volume of water from the corresponding depth before filling

them to the brim and tightly capping them. In many cases, samples have to be observed immediately *in vivo*, to determine motility and general appearance of the organisms.

### Chemical measurements

a) Oxygen (Winkler method). To check for correct functioning of the oxygen electrodes, Winkler titrations should be done periodically. This should be done especially at the depths of coexistence of oxygen and sulfide. The modification of INGVORSEN & JØRGENSEN (1979) has to be used in order to avoid interferences from sulfide.

b) Sulfide. Sulfide can be measured in a number of ways. A titration with acid iodine can be performed after precipitating the sulfide with  $\text{CdCl}_2$  or Zn acetate under alkaline conditions (GOLTERMAN *et al.*, 1978). This method is extremely simple and has the advantage of using some of the same solutions as the Winkler titration, but it can only be used at relatively high sulfide concentrations. The methylene blue colorimetric method provides a good alternative, especially for low concentrations of sulfide. Samples can be immediately precipitated as in the previous case and analyzed later in the laboratory (CLYNE, 1969; GOLTERMAN *et al.*, 1978) or the reaction can be performed directly in the field and the color read later in the laboratory. Finally, samples with sulfide can be mixed *in situ* with a highly alkaline antioxidant buffer (to prevent both oxidation and escape of the gas) and later determined with a silver-sulfide electrode (BAUMANN, 1974).

c) Nutrients. Phosphate is measured by the molybdate blue method (STRICKLAND & PARSONS, 1972; GOLTERMAN *et al.*, 1978). Sulfide can interfere with this method and give artificially high values. Samples can be bubbled with  $\text{N}_2$  to purge them of sulfide before the analysis. Care, however, should be taken when interpreting results. For the remaining nutrients conventional methods can be used (for example those in STRICKLAND & PARSONS, 1972; GOLTERMAN *et al.*, 1978). Samples for phosphate, nitrate, silicate, and DOC have to be filtered through Whatman GF/F filters (or similar filters) *in situ*, and biological activity stopped immediately by addition of appropriate fixatives (chloroform, boric acid or mercuric chloride for phosphate, nitrate and DOC respectively). Samples for nitrite determinations must be fixed in sulfanilamide acid reagent for posterior analysis in the laboratory by the Griess method (GOLTERMAN *et al.*, 1978).

d) Iron compounds. Samples for iron determinations can be taken in BOD bottles to guarantee protection from

oxygen, and fixed with sulfuric acid (1% final concentration). Ferrous iron is determined by the o-phenanthroline method. Total iron is determined by the same method after reduction of ferric to ferrous iron with ascorbic acid (GOLTERMAN *et al.*, 1978).

### Enumeration of organisms and pigment quantification

The abundance of picoplankton organisms (bacteria and cyanobacteria) and of small flagellates is determined by the acridine orange (either ZIMMERMANN & MEYER-REIL, 1974; or HOBBI *et al.*, 1977) or the DAPI epifluorescence methods (PORTER & FEIG, 1980), after fixation either with formalin or glutaraldehyde (2% final concentration buffered in Na-cacodylate, pH 6.8). The nanoplankton organisms

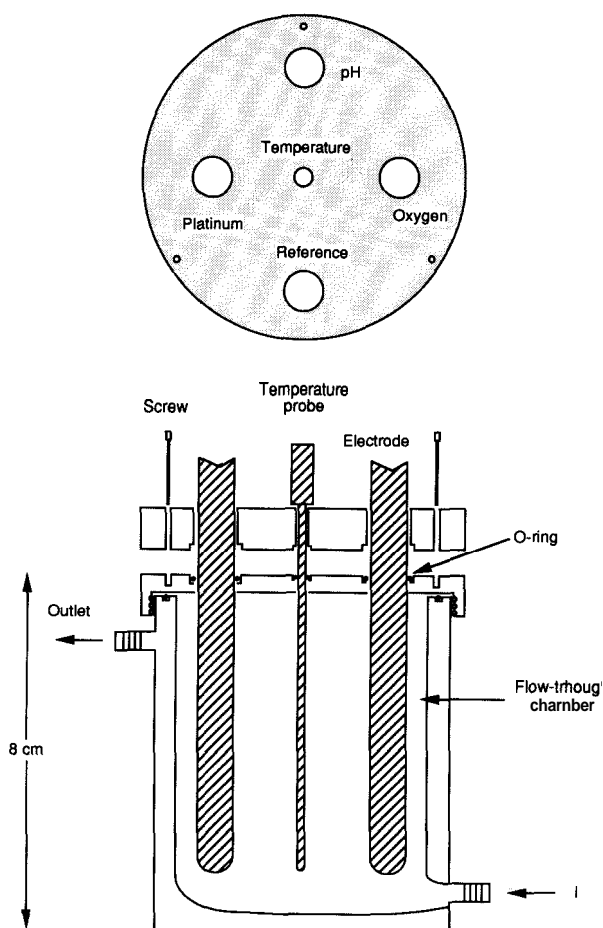


Figure 2. Flow-through chamber used to measure Eh, pH, oxygen and temperature *in situ*. Water from the conic sampler is directly pumped through the inlet and readings of the electrodes are recorded at the desired depth intervals. The upper portion of the drawing shows the lid from above.

(most phytoplankton and small ciliates) are usually fixed with Lugol and counted by the Utermohl technique (UTERMOHL, 1958) in a sedimentation chamber with an inverted microscope. Many of these organisms can be counted in chambers such as the Sedgwick-Rafter cell if their abundance is high enough or if a previous concentration step is performed. Rotifers and crustaceans usually have to be concentrated by filtration with nets of appropriate mesh size before counting them with an inverted microscope. These organisms are usually fixed with formalin to 4% final concentration.

Since many of the microorganisms at interfaces are phototrophic, it is important to determine the abundance of pigments. Chlorophylls, bacteriochlorophylls and carotenoids have taxonomic value and many times provide an easy-to-determine, synoptic view of the communities. Spectrophotometric determination of pigments is adequate for routine purposes. The coincidence of several absorption maxima at the same wavelengths, however, creates a problem when Chl *a* and Bchl *c* coexist. The chromatographic separation procedures are quite involved and time consuming and, thus, HPLC becomes the method of choice when characterizing a new system or performing detailed studies. We will summarize one spectrophotometric method and one HPLC procedure we have used successfully.

a) Spectrophotometric method. Known volumes from each depth are filtered on small pore size glass fiber filters. Pigments are extracted by placing the filters in 5 ml of 90% acetone supersaturated with MgCO<sub>3</sub> in screw-capped tubes, covered with aluminum foil to prevent degradation of pigments through exposure to light. The extraction proceeds at 4° C, in the dark, overnight, after which the tubes are centrifuged, part of the supernatant is carefully pipetted out with a micropipette and placed in quartz cuvettes. Absorption spectra are determined in a spectrophotometer between 350 and 850 nm. Chl *a* concentration is calculated with the trichromatic formula of JEFFREY & HUMPHREY (1975) with absorbance at 830 nm used to correct for background absorption because of the presence of bacteriochlorophyll *a*, which absorbs at 775 nm in acetone extracts. Bacteriochlorophylls (BChl) are determined with the equations of TAKAHASHI & ICHIMURA (1968), STANIER & SMITH (1960), and GLOE *et al.*, (1975).

b) HPLC method. Samples for HPLC analysis are pumped directly on a filtering system with Whatman GF/F filters. The filters are then immediately frozen in dry ice and kept frozen until the analysis is carried out. The method of MANTOURA & LLEWELLYN (1983) can be used with minor modifications. HURLEY & WATRAS (1991) and

KOVRTALS & STEENBERGEN (1985) have recently described HPLC protocols adequate for both algae and phototrophic bacteria. The filters are ground with a teflon-glass grinder in 90% acetone. Absorbance is measured at 440 nm, and fluorescence detection is used to aid in the identification of pigments. Peak areas are measured on the absorbance trace. If a diode array is available, each peak can be identified through its absorption spectrum.

For analysis, a 100-150 µl mixture of 4:1 pigment extract in 90% acetone and an ion-pairing solution, is injected into the chromatographic system. A gradient comprising some steps is run: from 100% eluent A (a highly polar solvent) to 100% eluent B (a highly non polar solvent). Organic solvents must be HPLC grade, and has to be purified (for example with a Milli-Q system, Millipore Corp.). Pigments are identified by comparing their retention time against chromatographic profiles of pure cultures of different microorganisms.

## RESULTS AND DISCUSSION

The systems studied were the karstic lakes in Eastern Spain (see fig. 1 in GUERRERO & PEDRÓS-ALIÓ, 1992). The different water bodies have been classified according to the mixing regime and the amount of dissolved sulfate in the water (table 1). We will examine the physicochemical stratification and the distribution of organisms in a few ecosystems chosen as representative of the different lake types in table 1.

### Physico-chemical stratification

The most striking feature of these lakes is the existence of a multilayered planktonic community with a high abundance of organisms in a thin portion of the water column, coincident with the zone of variation of the redox potential, which may coincide with a layer of oxygen and sulfide coexistence (Lake Cisó in fig. 3) or may span a zone without either oxygen or sulfide (Laguna de la Cruz in fig. 3). The depth at which the redoxcline and the associated layers of organisms are found varies with the time of the year and the vagaries of climate.

In meromictic lakes the redoxcline coincides with the depth of meromixis when the mixolimnion is not stratified. The depth of meromixis is characterized by an increase in temperature and conductivity, and only varies substantially under exceptional conditions (fig. 4). During summer thermal stratification, the redoxcline and the associated micro-

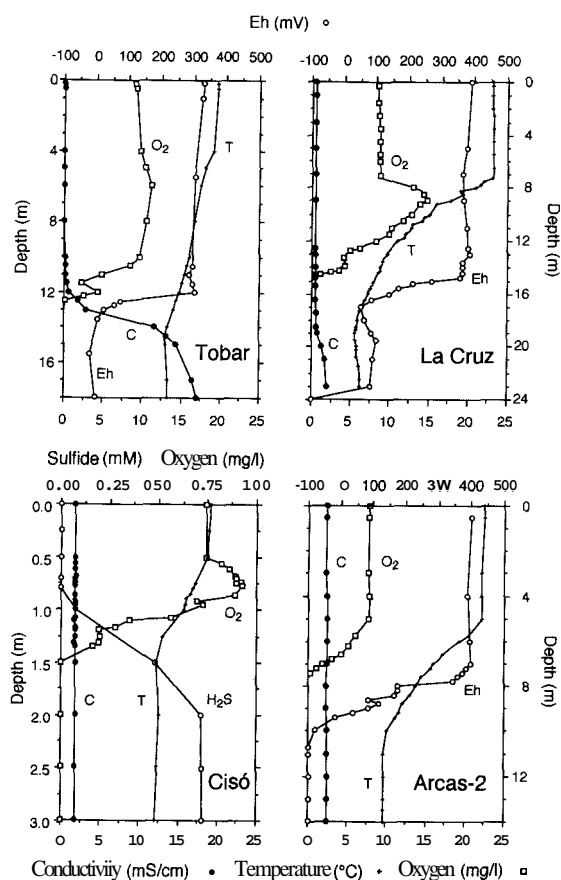


Figure 3. Vertical distribution of physico-chemical parameters in crenogenic meromictic Lake El Tobar (August 11, 1991), biogenic meromictic, low sulfide, Laguna de la Cruz (August 27, 1987), and holomictic, high sulfide Lakes Cisó (June 11, 1991) and Arcas-2 (August 28, 1987). Temperature (+), oxygen (O), conductivity (●) and Eh (○) have been plotted at the same scales in all panels except for Lake Cisó, where sulfide (O) is plotted instead of Eh, and where oxygen readings off the scale have to be multiplied times 10 to obtain actual values. Only the upper three meters are shown in Lake Cisó to show the detail obtained with the syringe sampler used between 0.5 and 1.5 m. The cone sampler was used in the other lakes, where many data points have been omitted to clarify the drawings.

bial populations do not always coincide with the depth of meromixis. In the more productive lakes studied, the redoxcline rises as the season advances, and may reach the thermocline towards the end of the summer. As the upper water is progressively mixed down in the fall, the redoxcline is pushed down with the thermocline until it reaches the depth of meromixis again.

Hardness of the water, which is an essential feature of karstic lakes, often gives peculiar optical properties to the water. In Laguna de la Cruz, with alkalinities over  $5 \text{ meq l}^{-1}$ , the blue-green water color turns Turkish blue and then

completely white in the last week of July or the first week of August every year. This "whiting" is due to calcium carbonate needle-shaped crystals, some forming aggregates. This phenomenon occurs suddenly and persists only for a week. Co-precipitation of phosphorous and decrease of light penetration causes a reduction of phytoplankton abundance and diversity. Secchi transparency during "whiting" in Laguna de La Cruz is only half a meter. Usually after a week it recovers back to the usual readings around 6 m. However, because of calcium carbonate precipitation and decrease of phytoplankton abundance, Secchi depth may reach 12 m about two weeks after the "whiting" phenomenon.

The extinction coefficient by layers in Laguna de la Cruz is high in the first meter layer due to its higher temperatures, which favor  $\text{CaCO}_3$  saturation, and to larger amounts of particles. The extinction coefficient is low (mean of  $0.2 \text{ m}^{-1}$ ) at intermediate depths, and rises enormously at the level of the redoxcline, because of the high abundance of microorganisms. In summer, light intensities from 1 to  $10 \mu\text{Einst. m}^{-2} \text{ s}^{-1}$  (approximately 0.1 and 1% of surface incident light respectively) reach these layers, where photosynthetic activity is substantial and usually higher than in the epilimnion.

Oxygen profiles in meromictic lakes are quite distinctive (figs. 3 and 4). They are both extremely clinograde and positively heterograde. There is a permanently anoxic layer at the bottom, segregated by a steep gradient. A very pronounced metalimnetic oxygen maximum is observed generally during the spring, with supersaturated values reaching up to 200% (about  $20 \text{ mg l}^{-1}$ , fig. 4). This oxygen maxima correspond to maxima of photosynthesis by phytoplankton and, thus, their exact depth changes with thermal stratification and plankton activities. Oxygen is trapped in the density gradient at the thermocline, where diffusion is additionally decreased by hydrostatic pressure and lack of turbulence. In the epilimnion, gas solubility decreases with increasing summer temperatures and diffuses to the atmosphere favored by turbulence. Thus the epilimnion has an homogeneous oxygen concentration close to the saturation value.

At the depth where  $\text{O}_2$  disappears, a shift in redox potential takes place (fig. 3). The redoxcline, therefore, appears slightly below the oxycline. Redox potential is lower at the end of the summer. In sulfate-rich lakes such as Lakes Cisó and Arcas-2 (fig. 3), high rates of sulfate reduction cause intense  $\text{H}_2\text{S}$  release, and the redox potential is much more negative than in waters with low sulfate concentration. In such lakes there is usually a zone of oxygen and sulfide coexistence.



Concentrations of many ions begin to increase at the redoxcline:  $\text{HCO}_3^-$ ,  $\text{Ca}^{2+}$ ,  $\text{SiO}_3^{2-}$ ,  $\text{Fe}^{2+}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NH}_4^+$  as well as  $\text{CO}_2$ ,  $\text{H}_2\text{S}$  and  $\text{CH}_4$ , which are the characteristic ions and gases in the monimolimnetic waters. In biogenic meromictic lakes (for example in Laguna de la Cruz) these gradients are maintained and enhanced by the action of the planktonic organisms and the accumulations of bicarbonates and iron in the monimolimnion are only possible through the biological activities, which modify the water pH and Eh and the concentration of reduced compounds.

In crenogenic meromictic Lake El Tobar, the oxygen distribution in the mixolimnion presents a general pattern similar to those in other meromictic lakes: a metalimnetic oxygen maximum during thermal stratification and oxygen exhaustion in the deep hypolimnion (fig. 3). A second relative oxygen maximum, located just at the beginning of the halocline is characteristic of this lake during spring and summer, due to the density gradient and an accumulation of phytoplankton at this depth. A permanent, very sharp, halocline separates the upper water layers from the monimolimnion, constituted by a brine mostly made of sodium chloride.

#### Phototrophic phytoplankton

Phytoplankton has been studied in Lake Banyoles (PLANAS, 1973), Lake Vilar (GARCIA DE EMILIANI,

1973), Lake Cisó (PEDRÓS-ALIÓ *et al.*, 1987; GASOL & PEDRÓS-ALIÓ, 1991; GASOL *et al.*, 1991a, 1991b, 1992), Lake Montcortés (CAMPS *et al.*, 1976; MODAMIO *et al.*, 1988), Lake Estanya (ÀVILA *et al.*, 1984), Laguna de la Cruz (ROJO & MIRACLE, 1987; DASI & MIRACLE, 1991) and Lake Arcas-2 (FINLAY *et al.*, 1991; VICENTE *et al.*, 1991). Here, we will summarize the common traits of vertical distribution and seasonal succession.

Phytoplankton is often rather poor in species in karstic lakes, and seasonal succession follows a rather regular pattern. In spring, the vertical mixing concurrent with the increase of insolation promotes a spring bloom of phytoplankton, especially of diatoms of the genus *Cyclotella*. In Lake Banyoles and Laguna de la Cruz the dominant species is *C. glomerata*. These diatoms tend to accumulate at the incipient thermocline in spring and form important metalimnetic maxima during early summer (until the beginning of August). These maxima are of the order of  $1$  to  $3 \times 10^3$  cells  $\text{ml}^{-1}$ . Although cells of *Cyclotella* spp. are always present, their numbers decrease during the last part of the summer ( $< 100$  cells  $\text{ml}^{-1}$ ). In summer a bloom of Chlorophyceae usually occurs in the mixolimnion of the more productive lakes studied. In Laguna de la Cruz, *Crucigenia rectangularis* forms a dense bloom in July ( $3$  to  $5 \times 10^3$  cells  $\text{ml}^{-1}$ ). Dominance is later shifted towards *Pedinomonas minor* in late summer, which reaches its maximal abundance ( $1$  to  $5 \times 10^3$  cells  $\text{ml}^{-1}$ ) just above the oxycline (fig. 5A). In

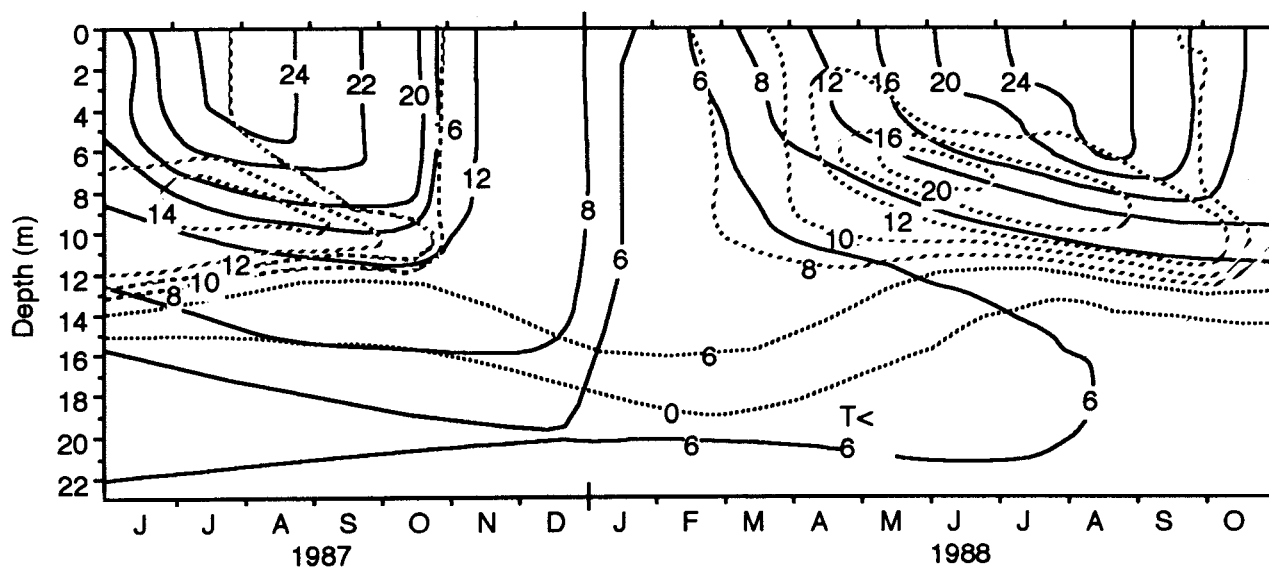


Figure 4. Vertical distribution of temperature ( $^{\circ}\text{C}$ , continuous lines) and oxygen ( $\text{mg l}^{-1}$ , discontinuous lines) through the seasons in Laguna de la Cruz.

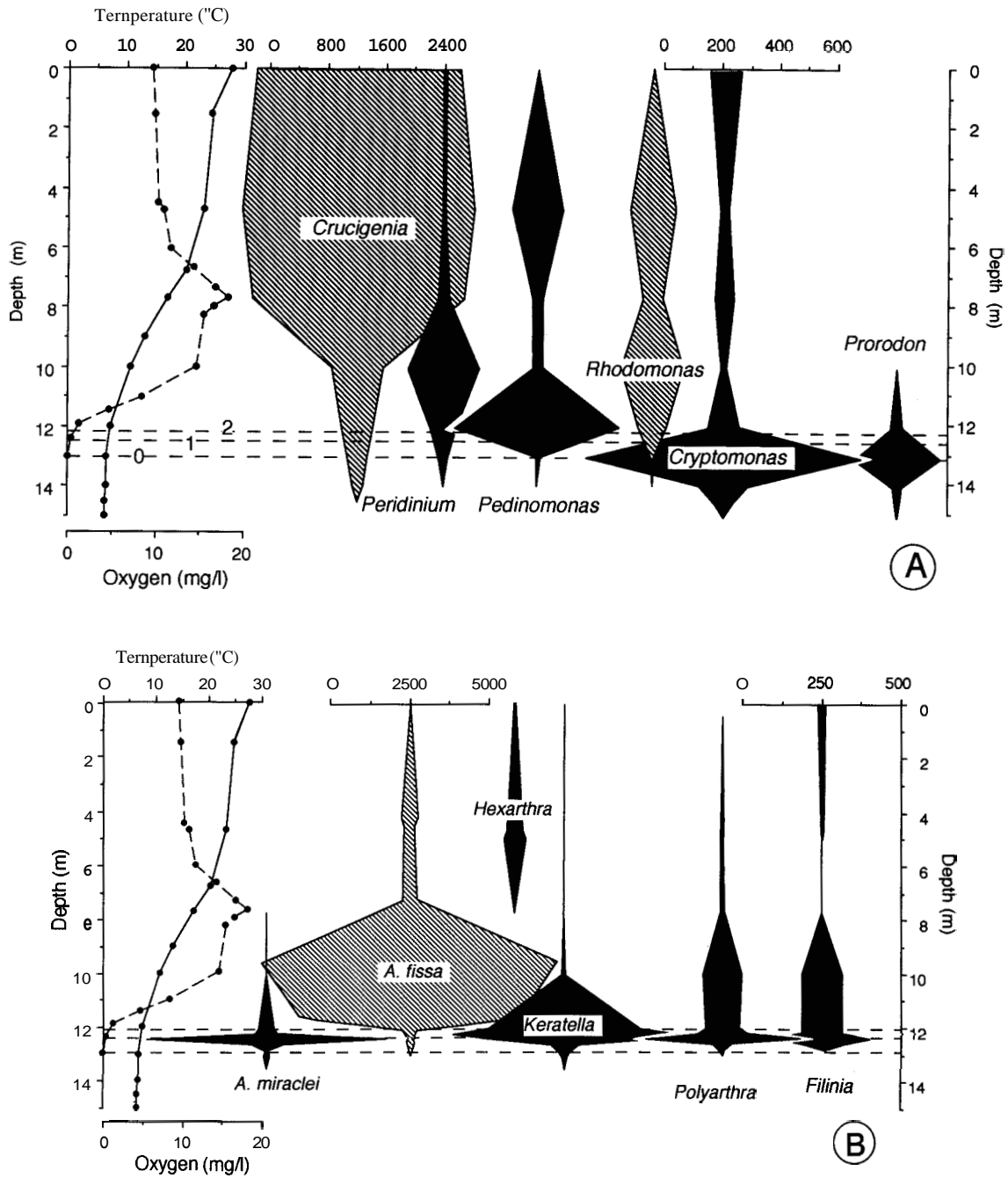


Figure 5. Vertical distribution of phytoplankton (A) and zooplankton (B) in Laguna de la Cruz on July 21, 1988. The three horizontal discontinuous lines indicate the oxygen concentration isopleths of 0, 1, and 2 mg l<sup>-1</sup>, respectively. Temperature (continuous line) and oxygen (discontinuous line) profiles are also shown. A) Use the 0 to 2400 cells ml<sup>-1</sup> scale for striped kites and the 0 to 600 cells ml<sup>-1</sup> scale for the black kites. *Crucigenia rectangularis* and *Rhodomonas lacustris* are found throughout the mixolimnion. *Peridinium*, *Pedinomonas minor*, three species of *Cryptomonas* and the *Chlorella*-bearing ciliate *Prorodon* present deep maxima. Note that the maximal abundance of *Cryptomonas* and *Prorodon* is found at the depth of 0 mg l<sup>-1</sup> oxygen concentration. B) Symbols are as in A). For *Anuraeopsis fissa* (striped kite) use the 0 to 5000 individuals l<sup>-1</sup> scale, and for the other rotifers (*H. mira*, *K. quadrata*, *F. hofmanni*, and *Polyarthra* sp.), black kites) use the 0 to 500 individuals l<sup>-1</sup> scale. Note vertical separation of the two species of *Anuraeopsis* and the depth of the maxima of most rotifers around the 1 mg l<sup>-1</sup> oxygen concentration. (Data from DASI & MIRACLE, 1991 and ARMENGOL et al., in press).

Lake Estanya the most important chlorophytes, are *Planktonema lauterhorni* ( $1 \times 10^4$  cells  $\text{ml}^{-1}$ ) and *Chodatella quadriseta* ( $0,5 \times 10^3$  cells  $\text{ml}^{-1}$ ). In Lake Cisó, *Crucigenia tetrapedia*, *Selenastrum capricornutum*, *Chlorella vulgaris* and *Hyaloruphidium contortum* usually appear in the epilimnion and later accumulate at the metalimnion.

Several species of *Peridinium* commonly appear in the summer metalimnion. Some of these (*P. inconspicuum*) are more or less permanent. *Peridinium* species present relative maxima (around 100 cells  $\text{ml}^{-1}$ ) at the incipient spring thermocline and at the deep metalimnion in summer. Another dinoflagellate, *Ceratium hirundinella*, appears usually in the epilimnion during the summer.

Many of the algae tend to concentrate at the oxycline during stratification. In Laguna de la Cruz, for example, total phytoplankton abundance is around 1000 cells  $\text{ml}^{-1}$  in winter throughout the water column. Around 6000 cells  $\text{ml}^{-1}$  can be found in the incipient thermocline in early spring and very low values through the rest of the vertical profile. During stratification, maxima appear at the oxycline with abundances between 5000 and 10000 cells  $\text{ml}^{-1}$  (fig. 5A). In meromictic Laguna de la Cruz, the community associated with the oxic/anoxic interface is mostly composed of three species of *Cryptomonas*: *Cryptomonas erosa*, *C. phaseolus* and *C. ovohata*, three of *Trachellomonas*, although only *Trachellomonas hispida* is common, three of *Astasia* and two of *Euglena*. The dominants are by far the species of *Cryptomonas*, which form rather dense and constant populations during stratification ( $1$  to  $4 \times 10^3$  cells  $\text{ml}^{-1}$ , fig. 5A). The peak of abundance is located at the depth of oxygen extinction which varies in Laguna de la Cruz between 18 m in winter and 15 to 13 m in summer (figs. 4 and 5A).

In Lake Cisó, more than 90% of the phytoplankton biomass is concentrated at the metalimnion during stratification (GASOL *et al.*, 1992). In this lake the oxycline coincides with the thermocline. Some species grow both at the epi- and metalimnion in early spring and then disappear from the epilimnion in early summer, leaving residual metalimnetic peaks (GASOL & PEDRÓS-ALIÓ, 1991). *Cryptomonas phaseolus*, on the other hand, grows in the metalimnion and remains active at this depth throughout stratification, with abundances up to  $10^5$  cells  $\text{ml}^{-1}$ . These development of oxyclinal population maxima of *Cryptomonas* are a characteristic feature of most karstic lakes examined. Small chlorophyte flagellates such as *Chlamydomonas* or *Pedinomonas* (fig. 5A) are commonly found above the layer of *Cryptomonas*.

In Lakes Montcortés (CAMPS *et al.*, 1976), Estanya (ÀVILA *et al.*, 1984), Lagunillo del Tejo (VICENTE &

MIRACLE, 1984) and Arcas-2 (VICENTE *et al.*, 1991; FINLAY *et al.*, 1991) *Cryptomonas* is found together with abundance maxima of *Oscillatoria*. These *Cryptomonas* and *Oscillatoria* populations must be adapted to low light conditions. Both have phycobilins as accessory pigments. These populations of *Cryptomonas* seem to tolerate the presence of sulfide, at least during the night, while the cyanobacteria seem to resist sulfide for longer periods of time (VICENTE *et al.*, 1991). In monomictic Lake Arcas-2, for instance, a dissolution lake on gypsum marl substrata, an anoxic hypolimnion develops during summer stagnation, from March to October. The oxyclinal community is formed by a peak of *Cryptomonas*, with abundances up to 17000 cells  $\text{ml}^{-1}$  in a thin water layer less than half a meter thick. Abundant *Oscillatoria* filaments (around 500  $\text{ml}^{-1}$ ) can be found together with the *Cryptomonas* layer. Layers of non filamentous cyanobacteria have been also described in the literature, such as *Synechococcus* in several lakes (KOHRTALS & STEENBERGEN, 1985; CRAIG, 1987). In Spanish lakes, however, we have only found a maximum of the orange pigmented *Gloeocapsa* in Lake El Tobar, just above the halocline.

Oxyclinal peaks of flagellates are common in stratified lakes throughout the world, for example *Scourfieldia caeca* in Tasmanian meromictic lakes (CROOME & TYLER, 1990; MIRACLE *et al.*, 1991), *Chrysophaerella longispira* in Canadian lakes (PIČEK *et al.*, 1984), or *Cryptomonas* in Finnish lakes (ARVOLA *et al.*, 1991) and mountain lakes in central Europe (GERVAIS, 1991; ROTT, 1988), among many other examples. These oxyclinal communities differ from the mixolimnetic communities in several respects. First, they are usually formed by only one or a few species. Second, they accumulate a tremendous amount of biomass. Third, they usually maintain a dynamic equilibrium throughout stratification, with low growth rates and reduced losses. Fourth, since predators seem inefficient at cropping this biomass, the carbon produced at the oxycline does not follow the traditional pathways to fishes and remains largely unexploited. Therefore, models of lake functioning, such as the PEG model of succession (SOMMER *et al.*, 1986) or empirical models of phytoplankton abundance and production (DILLON & RIGLER, 1974) are bound to fail when applied to lakes with important oxyclinal communities.

### Phototrophic bacterioplankton

The purple and green phototrophic sulfur bacteria are one of the best known features of meromictic and stratified lakes, because they build microstratified plates which are dense

enough to color the water green, brown or purple. GUERRERO *et al.*, (1987) summarized studies on the phototrophic sulfur bacteria of several Spanish lakes. ABELLÀ (1980), ABELLÀ *et al.*, (1980, 1981, 1985), GARCIA-GIL *et al.*, (1987, 1991) and MAS *et al.*, (1990) present annual cycles of these bacteria in lakes of the Banyoles karstic area, and VICENTE *et al.*, (1991) in Arcas-2. As a general feature Chlorobiaceae tend to be almost always present. During the summer and early autumn stratification period an important maximum of Chromatiaceae tends to develop above the Chlorobiaceae. Sometimes Chromatiaceae are present for a longer part of the year, but they do not develop dense peaks until complete stagnation of the lake. In Lake Cisó there is a shift from brown *Chlorobium phaeobacteroides* to green *Chlorobium limicola* when the maximum of Chromatiaceae is present (MONTESINOS *et al.*, 1983) because of the different absorption properties of the two organisms, one able to withstand the shading by Chromatiaceae and the other unable to use the remaining light wavelengths. The brown Chlorobiaceae are better adapted to low light intensities than the green Chlorobiaceae and, thus, predominate in lakes where sulfide and light meet at depth, or when large abundances of algae reduce light intensity.

In lakes where Chlorobiaceae are dominant gas-vacuolate species are often present, such as in Laguna de la Cruz, where *Pelodictyon clathratiforme* is the predominant phototrophic bacterium, presenting bacteriochlorophyll *d* concentrations up to 300 - 400  $\mu\text{g l}^{-1}$ . In this lake the dominant species of Chromatiaceae is *Amoebobacter*. Chromatiaceae, however, do not form spectacular peaks, since the amount of sulfide is low and the oxic-anoxic boundary is rather deep and, thus, receives too low light intensities. Lake Arcas-2, on the contrary, has enough  $\text{H}_2\text{S}$  reaching the illuminated interface to allow the development of a dense plate of *Chromatium weissii*, with Bacteriochlorophyll *a* concentrations up to 800  $\mu\text{g l}^{-1}$  (VICENTE *et al.*, 1991). *Amoebobacter* is also present albeit in smaller amounts. In Lake Cisó, *Amoebobacter* and *Chromatium minus* share dominance of the phototrophic bacterial guild (PEDRÓS-ALIÓ *et al.*, 1986; PEDRÓS-ALIÓ, 1989). Interestingly, all these Chromatiaceae abundant in nature have the carotenoid okenone as an accessory pigment (GUERRERO *et al.*, 1987; VICENTE *et al.*, 1991). The dynamics and ecology of phototrophic bacteria are more extensively treated in GUERRERO & PEDRÓS-ALIÓ (1992).

Opposite gradients of oxygen and reduced compounds intersect at the redoxcline (fig. 3). This provides a suitable environment for the development of different groups of

chemolithoautotrophic bacteria (SOROKIN, 1970; GORLENKO *et al.*, 1983). These bacteria fix  $\text{CO}_2$  (in the dark) as a source of carbon and use the oxidation of sulfide, ammonia or reduced iron as a source of electrons and energy. Bacteria able to oxidize thiosulfate have been found at the redoxcline in Lakes Cisó, Vilar and Estanya in abundances around  $10^4$  cells  $\text{ml}^{-1}$  (unpublished data of E. Ortega and J. Mas). Their activity has been determined by  $^{14}\text{CO}_2$  incorporation in the dark, and it has been found to be more important than photosynthesis in Lake Cisó and substantial in Lakes Vilar and Estanya (GARCIA-CANTIZANO *et al.*, 1987). The role of these bacteria seems, therefore, more important than previously thought.

### Zooplankton

The zooplankton in the mixolimnion of Spanish karstic lakes corresponds to their characteristically highly mineralized and oligo-mesotrophic waters. In general, crustaceans and rotifers are found in the same proportion with respect to the number of individuals, but a clear dominance of crustaceans (>75 %) is found when biomass is considered. On the other hand, rotifers are generally dominant at the oxycline of meromictic and stratified lakes, although many nauplii of copepods are also present. The abundance of some rotifers can reach up to  $4 \times 10^5$  individuals  $\text{l}^{-1}$  right at the oxycline, while mean abundance in the mixolimnion can be two to four orders of magnitude lower.

Studies of crustacean and rotifer zooplankton have been carried out in Lake Banyoles (MIRACLE, 1974, 1975, 1976; MIRACLE & ALFONSO, 1992), Lake Cisó (ALFONSO & MIRACLE, 1987; GASOL *et al.*, 1991a, 1991b, 1992), Lagunillo del Tejo, Laguna de la Cruz and Arcas-2 (MIRACLE & VICENTE, 1983; ESPARCIA *et al.*, 1991; FINLAY *et al.*, 1991; ARMENGOL-DIEZ *et al.*, 1992), Lake Montcortés (CAMPS *et al.*, 1976; MODAMIO *et al.*, 1988) and Lake Estanya (ÀVILA *et al.*, 1984). Ciliates have not received as much attention. Recent studies, however, have revealed the presence of very characteristic and abundant communities at the oxycline and the presence of anaerobic ciliates, able to tolerate sulfide, in the hypolimnia of Lake Arcas-2 (FINLAY *et al.*, 1991) and Lake Cisó (DYER *et al.*, 1986; GASOL *et al.*, 1991a, 1991b, 1992).

Another important zooplankton component is the larva of the dipteran *Chaoborus* (usually *Chaoborus flavicans*), a predator of the other members of the zooplankton, specially of copepods and some rotifers. *Chaoborus* performs important vertical migrations, inhabiting the bottom during the

day, but reaching high abundances in the whole vertical profile during the night (up to 3 individuals l<sup>-1</sup> in Laguna de la Cruz and 8 in Lake Cisó). *Chaoborus* larvae are the only components of the benthic fauna over most of the sediment surface.

a) Crustaceans.

The most common crustacean community consists of two cyclopoids: a small filter-feeder, generally *Tropocyclops prasinus* (in Lake Cisó *Thermocyclops dybowskii*) and a predator of the genus *Cyclops* (most commonly *Cyclops abyssorum*). These cyclopoids are generally present throughout the year with rather constant abundances. In the more complex systems additional cyclopoids such as *Diacyclops bicuspidatus* can be found.

Diaptomids have been only found in three out of over 30 surveyed karstic permanent water bodies in the Pre-Pyrenean, Banyoles and Cuenca regions. Diaptomids, however, are the most important crustacean components of temporary karstic lakes. When present in a permanent lake, Diaptomids tend to be the dominant organisms in the epilimnion, showing generally constant abundances through the year. The most characteristic species is *Arctodiaptomus salinus* (a smaller and paler ecotype than that inhabiting saline lakes). The presence of this organism is in accordance with the high mineralization of the waters. *A. salinus* (a circumMediterranean species) is found in Lake Banyoles and in Lake El Tobar. The third lake with Diaptomids is the Pre-Pyrenean Lake Basturs (Lleida), where *Eudiaptomus padanus* occurs, (described from lakes of the Pre-Alpine region).

The most frequent cladoceran assemblage consists of *Daphnia longispina* in autumn and spring and *Diaphanosoma brachyurum* in summer. During summer and autumn three smaller species are also commonly found: *Ceriodaphnia* (*Ceriodaphnia quadrangula* or *C. reticulata*), the more littoral *Bosmina longirostris* and the neustonic *Scapholeberis mucronata*.

b) Rotifers.

The abundance and composition of the rotifer assemblage changes markedly with time. Usually, around 20 species can be found coexisting in any one lake. Many of these constitute couples of species belonging to the same genus, which replace each other in time or become segregated in the vertical profile.

Four distinct groups of species can be usually found in summer (fig. 5B): (1) Epilimnetic species which are uniformly distributed through the epilimnion but are absent from the low oxygen hypolimnetic waters. Members of this group are *Synchaeta pectinata*, *Asplanchna girodi*, *Hexarthra fennica* and *Polyarthra remata*. (2) Species

present throughout the epilimnion, but showing a metalimnetic maximum. This group includes *Hexarthra mira*, *Keratella quadrata* and *Polyarthra vulgaris*. (3) Species which are widespread during mixing, but concentrate in the deepest microaerobic layers during the summer. Sometimes, these species show a distribution with two peaks: one at the thermocline and another at the oxycline. Species within this group are *Keratella cochlearis*, *Kellicotia longispina* (*K. longispina* is only present in Lakes Montcortés and Banyoles), *Anuraeopsis fissa* and *Polyarthra dolichoptera*. (4) Species which show a clear preference for the deep water layers. This group includes species which are exclusively found at the oxycline, such as *Filiniu hofmanni*, *Filinia longiseta longiseta*, and *Anuraeopsis miracleii* (the latter only in Laguna de la Cruz, fig. 5B). Other members of this group are species found in the whole vertical profile with an upper hypolimnetic maxima during the spring, but limited to the oxycline during the summer, such as *Filiniu terminalis*.

The rotifer species which show a preference for the oxycline are characteristically the same in most of the lakes studied. The genera *Filiniu*, *Polyarthra*, *Anuraeopsis* and *Keratella* are the more clearly associated with deep maxima (fig. 5B). *Filinia* and *Anuraeopsis* may have a diet with a large component consisting of bacteria and detritus. In Lake Cisó, for example, *A. fissa* has been experimentally shown to feed on both phototrophic purple bacteria and chemotrophic bacteria using fluorescently labeled bacteria as food. *Polyarthra*, on the other hand, has been shown to feed on the metalimnetic maximum of *Cryptomonas phaseolus* using radioactively labeled algae as food (MASSANA & PEDRÓS-ALIÓ, submitted). *Polyarthra*, thus, may preferentially feed on metalimnetic algae (*Cryptomonas*) and the more poliphagous *Keratella* could select the smaller flagellates (*Rhodomonas*, chryso- and chloroflagellates) but also use detritus and bacteria. The photosynthetic bacteria, however, do not seem to be a very important food source for rotifers, since the vertical distributions of predator and prey barely overlap. Although the rotifers can grow under microaerophilic conditions, the phototrophic bacteria occupy the uppermost layer with sulfide, and this gas seems to prevent the presence of metalimnetic rotifers. In Lake Cisó, the rotifer *Mytilina compressa* has been found in the sulfide-rich hypolimnion (ALFONSO & MIRACLE, 1987), where it has been shown to feed on both purple phototrophic and chemotrophic bacteria (MASSANA & PEDRÓS-ALIÓ, unpublished).

The vertical distribution of pairs of congeneric species is fairly interesting and characteristic. Generally, there is

a stenoic species showing a very narrow and restricted occurrence at the oxycline, and a eurioic species. The latter may be differentiated into ecotypes and easily dominates over the stenoic species when stratification conditions change. The distribution of *Filinia* species has recently received considerable attention (RUTTNER-KOLISKO, 1989; HOF-MANN, 1982; SCHABER & SCHRIMPF, 1984). Careful taxonomic studies have revealed that the forms developing near the oxycline belong to a new species described as *Filinia hofmanni* (KOSTE, 1980). *F. hofmanni* is almost exclusively found at the oxycline of lakes. In Lake Banyoles *F. hofmanni* was only found in the basins that become anoxic (mostly in meromictic basin III). *F. hofmanni* has also been found in great numbers in Lake Arcas-2 and at lesser abundances in Laguna de la Cruz, where it alternates dominance in the oxycline with *A. miraclei* and *A. fissa* in different years (fig. 5B). *F. terminalis* is more widely distributed, and presents oxyclinal maxima slightly above those of *F. hofmanni* when both species co-occur.

The differential vertical distribution of *Keratella* species in stratified lakes is also well known (LARSSON, 1971; HOFMANN, 1985, 1987; RUTTNER-KOLISKO, 1975, 1977; MIKSCHI, 1989). *Keratella hiemalis* is frequently restricted to the oxycline layers, but it is not present in Spanish karstic lakes. Its role in the microaerophilic layers may be taken over by *Keratella cochlearis* (Lake Banyoles) or by *Keratella quadrata* (Lakes Montcortés, Cisó and Laguna de la Cruz). These species show a distribution with two peaks: one at the epi-metalimnion boundary and another at the oxycline.

A similar case seems to be true for *Anurueopsis*. A new species from Laguna de la Cruz, *A. miraclei*, has been recently described (KOSTE, 1991) as an exclusively oxyclinal species (fig. 5B). This species coexists with the more widely distributed *A. fissa*, which can also present maxima at the oxycline (ARMENGOL *et al.*, 1992). In Lake Banyoles, where *A. miraclei* was not present, *A. fissa* showed a distribution with two peaks (MIRACLE, 1976; MIRACLE & ALFONSO, 1992). These different peaks could corres-

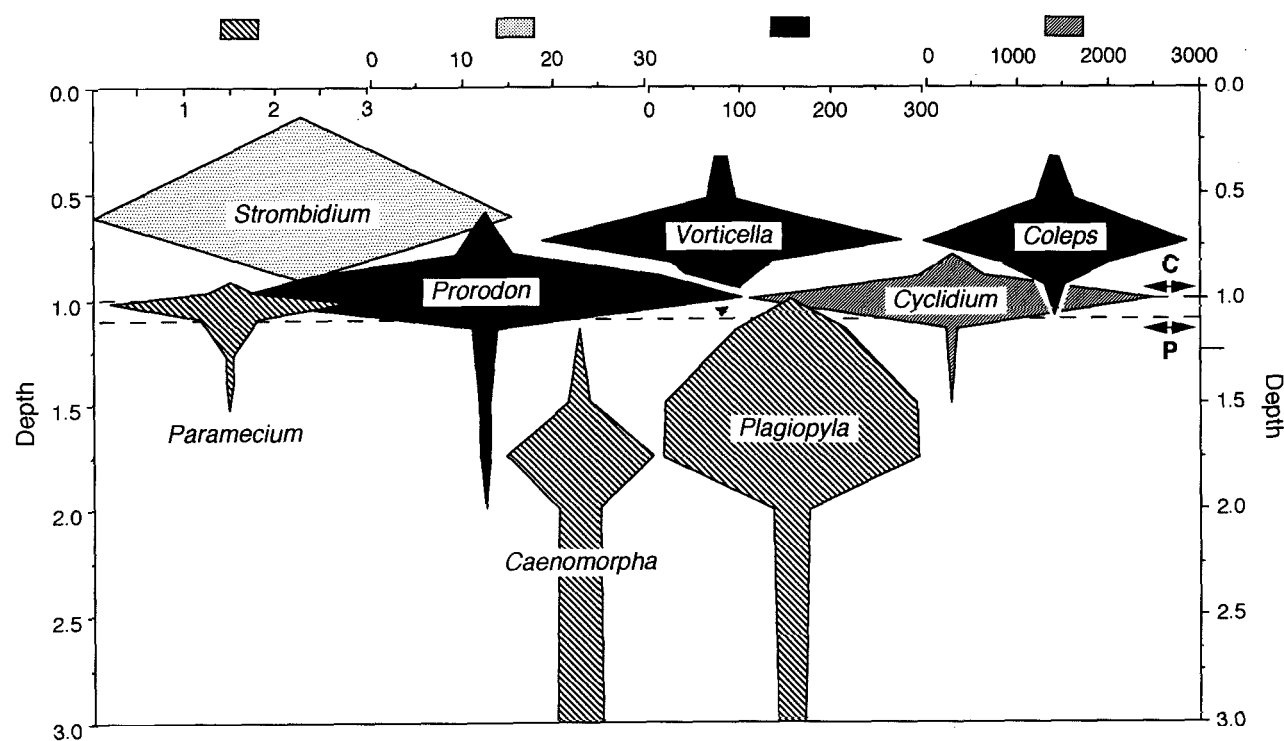


Figure 8. Vertical distribution of some ciliates in Lake Cisó (June 11, 1991). The two horizontal discontinuous lines enclose the region of sulfide and oxygen coexistence. The double arrows labeled C and P indicate, respectively, the depths of the *Cryptomonas phaseolus* and purple phototrophic bacteria (*Chromatium minus* and *Amoebobacter M3*) peaks of abundance. Note the four different scales for ciliates with differently shaded kites: *Paramecium*, *Plagiopyla* and *Caenomorpha* use the 0-3 ciliates  $ml^{-1}$  scale; *Strombidium* the 0-30 ciliates  $ml^{-1}$  scale; *Prorodon*, *Coleps* and *Vorticella* the 0-300 ciliates  $ml^{-1}$  scale; and *Cyclidium* the 0-3000 ciliates  $ml^{-1}$  scale. (Unpublished data of R. Massana).

pond to different ecotypes within the same species. In stratified lakes, a subdivision of rotifer populations of a genus into species and probably of a species into ecotypes clearly occurs. This differentiation matches the vertical environmental heterogeneity.

c) Ciliates.

Ciliate distribution has been studied in Lakes Cisó (fig. 6) and Arcas-2. The ciliates of karstic lakes can be divided into three separate communities (DYER et al., 1986; FINLAY et al., 1991): 1) an epilimnetic assemblage of aerobic, cosmopolitan ciliates such as *Vorticella*, *Paramecium*, *Stentor*, *Strombidium* and others. This assemblage includes both algivorous and bacterivorous ciliates. 2) an oxyclinal assemblage formed by sharply stratified maxima of algivorous ciliates with endosymbiotic *Chlorella* cells such as *Coleps hirtus* (ESTEVE et al., 1988) or *Prorodon* (see fig. 5A and fig. 6). And 3) an anaerobic assemblage constituted by sulfide-tolerant, bacterivorous ciliates such as *Metopus* es, *Plagiopyla ovata* or *Caenomorpha medusula*. Some of these anaerobic ciliates have endosymbiotic methanogenic bacteria (FINLAY et al., 1991). In addition, scuticociliates, e.g. *Cyclidium*, are found either in the oxycline or the monimolimnion. More details about the feeding activities of these ciliates can be found in GUERRERO & PEDRÓS-ALIÓ (1992) and FINLAY et al. (1991).

## CONCLUSION

Karstic lakes present accumulations of organisms at depth. The comparison of different kinds of meromictic and holomictic stratified lakes (fig. 3) has allowed to identify the redoxcline as the most important environmental factor for the organization of the community. In some cases the redoxcline may coincide with a thermocline, a chemocline or a halocline, but not necessarily. The organisms found in different lakes usually belong to the same genera and many times to the same species. Usually, one can find large abundances of copepod nauplii (especially *T. prasinus*), microaerophilic rotifers (*Anuraeopsis*, *Filinia*), flagellated algae (*Cryptomonas*) and in some lakes also cyanobacteria (*Oscillatoria*) at the oxic-anoxic boundary. Characteristic genera of ciliates are also associated with these interfaces (*Coleps*, *Prorodon*, and anaerobic ciliates), where purple and green sulfur bacteria form abundance maxima. The same components have been found in most lakes studied showing that this community is widespread and quite different from the epilimnetic community. Moreover, the deep living community persists throughout the

stratification period without much changes. These deep living communities, therefore, deserve more study before current models of trophic webs, nutrient cycling, and succession can be generalized to all aquatic communities.

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## REFERENCES

- ABELLA, C.A., 1980. Dinámica poblacional comparada de bacterias fototróficas planctónicas. Ph.D. Thesis. Autonomous University of Barcelona.
- ABELLÀ, C.A., E. MONTESINOS, & R. GUERRERO, 1980. Field studies on the competition between purple and green sulfur bacteria for available light. (lake Sisó, Spain). *Developments in Hydrobiology*, 3: 173-181.
- ABELLÀ, C.A., E. MONTESINOS & R. GUERRERO, 1981. Dinàmica i estratificació de poblacions bacterianes fototròfiques en una cubeta meromíctica del llac de Banyoles. *Bull. Soc. Catalana Biol.*, 5-7: 11-27.
- ABELLÀ, C.A., E. MONTESINOS & J. TURET, 1985. Colonization and dynamics of phototrophic bacteria in a recently formed lagoon in Banyoles karstic area (Girona, Spain). *Scientia gerundensis*, 10: 33-49.
- ALFONSO, M.T. & M.R. MIRACLE, 1987. Variación temporal de las poblaciones zooplanctónicas de la laguna anóxica del Cisó (Gerona). *Limnetica* 3: 167-177.
- ARMENGOL-DÍAZ, J., A. ESPARCIA, E. VICENTE & M.R. MIRACLE, 1992. Vertical distribution of planktonic rotifers in a karstic meromictic lake. *Hydrobiologia*, in press.
- ARVOLA, L., A. OJALA, F. BARBOSA & S. I. HEANEY, 1991. Migration behavior of three cryptophytes in relation to environmental gradients: an experimental approach. *British Phycol. Jr.*, 26: 361-373.
- AVILA, A., J.L. BURRELL, A. DOMINGO, E. FERNÁNDEZ, J. GODALL & J.M. LLOPART, 1984. Limnología del Lago Grande de Estanya. *Oecol. aquat.* 7: 3-24.
- BAKER, A. L., K. K. BAKER & P. A. TYLER, 1985. A family of pneumatically-operated thin layer samplers for

- replicate sampling of heterogeneous water columns. *Hydrobiologia*, 122: 207-211.
- BAUMANN, E.W., 1974. Determination of parts per billion of sulfide in water with sulfide selective electrode. *Anal. Chem.*, 46: 1345-1347.
- BJØRNSSEN, P.K. & T.G. NIELSEN, 1991. Decimeter scale heterogeneity in the plankton during a pycnocline bloom of *Gyrodinium aureolum*. *Mar. Ecol. Prog. Ser.*, 73: 263-267.
- CAMPS, J., I. GONZALVO, J. GÜELL, P. LÓPEZ, A. TEJERO, X. TOLDRÀ, F. VALLESPINÓS & M. VICENS, 1976. El lago de Montcortés, descripción de un ciclo anual. *Oecol. aquat.*, 2: 99-110.
- CLYNE, J.B., 1969. Spectrophotometric determination of hydrogen sulfide in natural waters. *Limnol. Oceanogr.*, 14: 454-458.
- CRAIG, S.R., 1987. The distribution and contribution of picoplankton to deep photosynthetic layers in some meromictic lakes *Acta Acad. Aboensis*, 47: 55-81.
- CROOME, R.L. & P.A. TYLER, 1990. Biological studies of meromictic lakes. *Limnology in Australia*, pp. 113-130.
- DASI, M.J. & M.R. MIRACLE, 1991. Distribución vertical y variación estacional del fitoplancton de una laguna cárstica meromíctica, la Laguna de la Cruz (Cuenca, España). *Limnetica*, 7: 37-59.
- DILLON, P. J. & F. H. RIGLER, 1974. The phosphorus-chlorophyll relationship in lakes. *Limnol. Oceanogr.*, 19: 767-773.
- DOMÍNGUEZ-PLANELLA, A., X.G. RIERA, L. POLO & C.A. ABELLÀ, 1987. Interrelación entre el mixolimnion y el monimolimnion en la laguna costera La Massona (Gerona). *Limnetica*, 3: 219-225.
- DYER, B.B., N. GAJU, C. PEDRÓS-ALIÓ, I. ESTEVE & R. GUERRERO, 1986. Ciliates from a freshwater sulfidum. *Biosystems*, 19: 127-135.
- ESPARCIA, A., J. ARMENGOL, E. VICENTE & M.R. MIRACLE, 1991. Vertical distribution of *Anuraeopsis* species as related to oxygen depletion in two stratified lakes. *Verh. Internat. Verein. Limnol.*, 24: 2745-2749.
- ESTEVE, I., J. MIR, N. GAJU, H. MCKHANN & L. MARGULIS, 1988. Green endosymbiont of *Coleps* from Lake Cisó identified as *Chlorella vulgaris*. *Symbiosis*, 6: 197-210.
- FINLAY, B.J., K.J. CLARKE, E. VICENTE & M.R. MIRACLE, 1991. Anaerobic ciliates from a sulphide-rich solution lake in Spain. *Europ. J. Protistol.*, 27: 148-159.
- GARCÍA-CANTIZANO, J., J.M. GASOL & C. PEDRÓS-ALIÓ, 1987. Producción primaria por fototrofia y por quimiolitotrofia en la laguna de Cisó. *Actas IV Cong.Esp. Limnología (Sevilla)*, pp. 75-84.
- GARCÍA DE EMILIANI, M.O., 1973. Fitoplancton de la laguna del Vilà (Gerona). *Oecol. aquat.*, 1: 107-155.
- GARCÍA-GIL, L.J., R.C. BRUNET & C.A. ABELLÀ, 1987. Incidencia de la inestabilidad de la meromixis en Banyoles IV (Lago de Banyoles, Gerona) en la dinámica poblacional de bacterias fototróficas del azufre. *Actas IV Cong.Esp. Limnología (Sevilla)*, pp. 85-94.
- GARCÍA-GIL, L.J., L. SALA-GENOHER & C.A. ABELLÀ, 1991. Dynamics of phototrophic bacteria in relation to iron cycle in a meromictic basin of Lake Banyoles. *Verh. Internat. Verein. Limnol.*, 24: 1016-1019.
- GASOL, J.M., J. MAS, C. PEDRÓS-ALIÓ & R. GUERRERO, 1990. Ecología microbiana y limnología en la laguna Cisó: 1976-1989. *Scientia gerundensis*, 16: 155-178.
- GASOL, J.M. & C. PEDRÓS-ALIÓ, 1991. On the origin of deep algal maxima: the case of Lake Cisó. *Verh. Internat. Verein. Limnol.*, 24: 1024-1028.
- GASOL, J.M., J. GARCÍA-CANTIZANO, R. MASSANA, F. PETERS, R. GUERRERO & C. PEDRÓS-ALIÓ, 1991a. Diel changes in the microstratification of the metalimnetic community in Lake Cisó. *Hydrobiologia*, 211: 227-240.
- GASOL, J.M., R. GUERRERO & C. PEDRÓS-ALIÓ, 1991b. Seasonal variations in size structure and prokaryotic dominance in sulfurous Lake Cisó. *Limnol. Oceanogr.*, 36: 860-872.
- GASOL, J.M., F. PETERS, R. GUERRERO & C. PEDRÓS-ALIÓ, 1992. Community structure in Lake Cisó: Biomass allocation to trophic groups and differing patterns of seasonal succession in the meta- and epilimnion. *Arch. Hydrobiol.*, 123: 275-303.
- GERVAIS, F., 1991. Which factors control seasonal and spatial distribution of phytoplankton species in Schlachtensee (Berlin, F.R.G.). *Arch. Hydrobiol.*, 121: 43-65.
- GLOE, A., N. PFENNIG, H. BROCKMANN & W. TROWITZSCH, 1975. A new bacteriochlorophyll from brown-coloured Chlorobiaceae. *Arch. Microbiol.*, 102: 103-109.
- GOLTERMAN, H.L., R.S. CLYMO & M.A.M. OHNSTAD, 1978. Methods for physical and chemical analysis of fresh waters. IBP Handbook n°8. Blackwell Sci. Pub., Oxford, U.K.
- GORLENKO, V.M., G.A. DUBININA & S.I. KUSNETSOV, 1983. The ecology of aquatic microorganisms. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, 252 pp.
- GUERRERO, R. & C.A. ABELLÀ, 1978. Dinámica espaciotemporal de las poblaciones bacterianas fotosintéti-



cas en una laguna anaerobia de aguas sulfurosas. *Oecol. aquat.*, 3: 193-205.

GUERRERO, R., C.A. ABELLÀ & M.R. MIRACLE, 1978. Spatial and temporal distribution of bacteria in a meromictic karstic lake basin: relationship with physico-chemical parameters and zooplankton. *Verh. Internat. Verein. Limnol.*, 20: 2264-2274.

GUERRERO, R., E. MONTESINOS, I. ESTEVE & C.A. ABELLÀ, 1980. Physiological adaptation and growth of purple and green sulphur bacteria in a meromictic lake (Vilà) as compared to a holomictic lake (Cisó). *Developmentals in Hydrobiology*, 3: 161-171.

GUERRERO, R., E. MONTESINOS, C. PEDRÓS-ALIÓ, I. ESTEVE, J. MAS, H. VAN GEMERDEN, P.A.G. HOFMAN & J.F. BAKKER, 1985. Phototrophic sulfur bacteria in two Spanish lakes: Vertical distribution and limiting factors. *Limnol. Oceanogr.*, 30: 919-931.

GUERRERO, R., C. PEDRÓS-ALIÓ, I. ESTEVE, J. MAS, D. CHASE & L. MARGULIS, 1986. Predatory prokaryotes: predation and primary consumption evolved in bacteria. *Proc. Natl. Acad. Sci. USA*, 83: 2138-2142.

GUERRERO, R., C. PEDRÓS-ALIÓ, I. ESTEVE & J. MAS, 1987. Communities of phototrophic sulfur bacteria in lakes of the Spanish Mediterranean region. *Acta Acad. Aboensis*, 47: 125-151.

GUERRERO, R. & C. PEDRÓS-ALIÓ, 1992. The microbial component in Spanish aquatic ecosystems. In C. Montes & C. Duarte (eds.) *Limnology in Spain*, Limnetica, 8.

HOBBIE, J.E., R.J. DALEY & S. JASPER, 1977. Use of Nuclepore filters for counting bacteria by epifluorescence microscopy. *Appl. Environ. Microbiol.*, 33: 1225-1228.

HOFMANN, W. 1982. On the coexistence of two pelagic *Filinia* species (Rotatoria) in Lake PlüBsee. I. Dynamics of abundance and dispersion. *Arch. Hydrobiol.*, 95: 125-137.

HOFMANN, W. 1985. Dynamics of vertical zooplankton community in the PlüBsee: Cluster analysis. *Verh. Internat. Verein. Limnol.*, 22: 2983-2986.

HOFMANN, W. 1987. Population dynamics of hypolimnetic rotifers in the PlüBsee (North Germany). *Hydrobiologia*, 147: 197-201.

HURLEY, J.P. & C.J. WATRAS, 1991. Identification of bacteriochlorophylls in lakes via reverse-phase HPLC. *Limnol. Oceanogr.*, 36: 307-314.

INGVORSEN, K. & B. B. JØRGENSEN, 1979. Combined measurement of oxygen and sulfide in water samples. *Limnol. Oceanogr.*, 24: 390-393.

JEFFREY, S.W. & G.F. HUMPHREY, 1975. New spectrophotometric equations for determining chlorophylls *a*, *b*,

*c*<sub>1</sub> and *c*<sub>2</sub> in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen*, 167: 191-194.

JØRGENSEN, B.B., J.G. KUENEN & Y. COHEN, 1979. Microbial transformations of sulfur compounds in a stratified lake (Solar Lake, Sinai). *Limnol. Oceanogr.*, 28: 1075-1093.

KORTHALS, H.J. & C.L.M. STEENBERGEN, 1985. Separation and quantification of pigments from natural phototrophic microbial populations. *FEMS Microbiol. Ecol.*, 31: 177-185.

KOSTE, W., 1980. Über zwei Plankton-Radertiergattungen *Filinia australensis* n. sp. und *Filinia hofmanni* n. sp., mit Bemerkungen zur Taxonomie der *longiseta* - *terminalis* Gruppe. Genus *Filinia* Bory de St. Vincent, 1824, Familie *Filiniidae* Bartos 1959 (Ubeordnung Monogononta). *Arch. Hydrobiol.*, 90: 230-256.

KOSTE, W., 1991. *Anuraeopsis miraclei*, a new planktonic rotifer species in karstic lakes of Spain. *Hydrobiologia*, 209: 169-173.

LARSSON, P., 1971. Vertical distribution of planktonic rotifers in a meromictic lake. *Norw. J. Zool.*, 19: 47-75.

MANTOURA, R.F.C. & C.A. LLEVELLYN, 1983. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography. *Anal. Chim. Acta*, 151: 297-314.

MAS, J., C. PEDRÓS-ALIÓ & R. GUERRERO, 1990. *In situ* specific loss and growth rates of purple sulfur bacteria in Lake Cisó. *FEMS Microbiol. Ecol.*, 73: 271-281.

MIKSCHI, E., 1989. Rotifer distribution in relation to temperature and oxygen content. *Hydrobiologia*, 186/187: 209-214.

MIRACLE, M.R., 1974. Niche structure in fresh water zooplankton. A principal components approach. *Ecology*, 55: 1306-1316.

MIRACLE, M.R., 1975. Segregation of zooplankton populations in several depressions within one lake basin. *Verh. Internat. Verein. Limnol.*, 19: 1140-1149.

MIRACLE, M.R., 1976. Distribución en el espacio y en el tiempo de las especies del zooplancton del lago de Banyoles. ICONA Monografías, Ministerio Agricultura y Pesca. 5: 1-270.

MIRACLE, M.R. & E. VICENTE, 1983. Vertical distribution and rotifer concentrations in the chemocline of meromictic lakes. *Hydrobiologia*, 104: 259-267.

MIRACLE, M.R. & E. VICENTE, 1985. Phytoplankton and photosynthetic sulphur bacteria production in the meromictic coastal lagoon of Cullera (Valencia, Spain). *Verh. Internat. Verein. Limnol.*, 22: 2214-2220.

- MIRACLE, M.R. & M.T. ALFONSO, 1992. Rotifer vertical distribution in a meromictic basin of Lake Banyoles (Spain). *Hydrobiologia*, (in press).
- MIRACLE, M.R., E. VICENTE, R.L. CROOME & P.A. TYLER, 1991. Microbial microcosms of the chemocline of a meromictic lake in relation to changing levels of PAR. *Verh. Internat. Verein. Limnol.*, 24: 1139-1144.
- MITCHELL, J. G. & J.A. FUHRMAN, 1989. Microdistribution of heterotrophic bacteria. *Mar. Ecol. Prog. Ser.*, 53: 141-148.
- MODAMIO, X., V. PÉREZ & F. SAMARRA, 1988. Limnología del lago de Montcortès (ciclo 1978-79) (Pallars Jussà, Lleida). *Oecol. aquat.*, 9: 9-17.
- MONTESINOS, E., R. GUERRERO, C. ABELLÀ & I. ESTEVE, 1983. Ecology and physiology of the competition for light between *Chlorobium limicola* and *Chlorobium phaeobacteroides* in natural habitats. *Appl. Environ. Microbiol.*, 46: 1007-1016.
- MOYA, G., G. RAMON, A. MARTÍNEZ TABERNER, V. FORTEZA, C. PICÓ, C. PONSELL, R. ROSSELLÓ & M.A. SOBERATS, 1987. Limnology of a meromictic coastal lagoon, L'Estany Cibollar (Majorca, Balearic Islands). *Limnetica*, 3(2): 255-262.
- PLANAS, M.D., 1973. Composición, ciclo y productividad del fitoplancton del lago de Banyoles. *Oecol. aquat.*, 1: 3-106.
- PEDRÓS-ALIÓ, C., 1989. Towards an autecology of bacterioplankton. In: U. Sommer (ed.) *Plankton Ecology. Succession in Plankton Communities*. 297-336. Springer-Verlag, Berlin.
- PEDRÓS-ALIÓ, C. & M.M. SALA, 1990. Microdistribution and diel vertical migration of flagellated vs. gas-vacuolate purple sulfur bacteria in a stratified water body. *Limnol. Oceanogr.*, 35: 1637-1644.
- PEDRÓS-ALIÓ, C., J.M. GASOL & R. GUERRERO, 1986. Microbial ecology of sulfurous lake Cisó. In: *F. Megusar & M. Gantar (Eds.). Perspectives in Microbial Ecology*: 638-643. Slovenian Soc. Microbiol., Ljubljana, Yugoslavia.
- PEDRÓS-ALIÓ, C., J.M. GASOL & R. GUERRERO, 1987. On the ecology of a *Cryptomonas phaseolus* population forming a metalimnetic bloom in Lake Cisó, Spain: Annual distribution and loss factors. *Limnol. Oceanogr.*, 32: 285-298.
- PICK, F. R., C. NALEWAJKO & D. R. S. LEAN, 1984. The origin of a metalimnetic chrysophyte peak. *Limnol. Oceanogr.*, 29: 125-134.
- PORTER, K.G. & Y.S. FEIG, 1980. The use of DAPI for identification and enumeration of bacteria and blue-green algae. *Limnol. Oceanogr.*, 25: 943-948.
- RIERA, X G. & C.A. ABELLÀ, 1986. Dinàmica anual de les poblacions de bacteris fototrofs de la llacuna costanera de La Massona (Alt Empordà, Girona). *Scientia gerundensis*, 12: 35-48.
- RIERA, X G. & C.A. ABELLÀ, 1991. Limnological cycle of the coastal lagoon La Massona (Girona, NE Spain). Origin, dynamics and influence of the sporadic seawater infalls on the meromixis. *Verh. Internat. Verein. Limnol.*, 24: 1029-1031.
- ROJO, C. & M.R. MIRACLE, 1987. Poblaciones fitoplanctónicas de la Laguna de la Cruz (Cuenca), una laguna cárstica meromíctica. *Actas IV Simp. Botanica Cryptogamica*, Granada (Spain): 119-135.
- ROJO, C. & M.R. MIRACLE, 1989. Phytoplankton fluctuations during an annual cycle in the coastal lagoon of Cullera (Spain). *Int. Revue ges. Hydrobiol.*, 74:179-194.
- ROTT, E., 1988. Some aspects of the seasonal distribution of flagellates in mountain lakes. *Hydrobiologia*, 161: 159-170.
- RUTTNER-KOLISKO, A., 1975. The vertical distribution of planktonic rotifers in a small Alpine lake with a sharp oxygen depletion (Lunzer Obersee). *Verh. Internat. Verein. Limnol.*, 19: 1286-1294.
- RUTTNER-KOLISKO, A., 1977. Population dynamics of rotifers as related to climatic conditions in Lunzer Obersee and Untersee. *Arch. Hydrobiol., Beinh. Ergebn. Limnol.*, 8: 88-93.
- RUTTNER-KOLISKO, A., 1989. Problems in taxonomy of rotifers, exemplified by the *Filinia longiseta - terminalis* complex. *Hydrobiologia*, 186/187: 291-298.
- SCHABER, P. & A. SCHRIMPF, 1984. On morphology and ecology of the *Filinia terminalis - longiseta* group (Rotatoria) in Bavarian and Tyrolean lakes. *Arch. Hydrobiol.*, 101: 247-257.
- SOMMER, U., Z. M. GLIWICZ, W. LAMPERT & A. DUNCAN, 1986. The PEG-model of seasonal distribution of planktonic events in freshwaters. *Arch. Hydrobiol.*, 106: 433-471.
- SOROKIN, Y.I., 1970. Interrelation between sulphur and carbon turnover in meromictic lakes. *Arch. Hydrobiol.*, 66: 391-446.
- STANIER, R.Y. & J.H.C. SMITH, 1960. The chlorophylls of green bacteria. *Biochim. Biophys. Acta*, 41: 478-484.
- STRICKLAND, J. D. H. & T. R. PARSONS, 1972. A practical handbook of seawater analysis. *Bull. Fish. Res. Board Canada*, 167.
- TAKAHASHI, M. & S. ICHIMURA, 1968. Vertical distribution and organic matter production of phototrophic sulfur bacteria in Japanese lakes. *Limnol. Oceanogr.*, 13: 644-655.

UTERMÖHL, H., 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt. Int. Verein. Limnol.*, 9: 1-38.

VICENTE, E., & M.R. MIRACLE, 1984. Distribution of photosynthetic organisms in a temporal stratified karstic pond near Cuenca (Spain). *Verh. Internat. Verein. Limnol.*, 22: 1504-1710.

VICENTE, E., & M.R. MIRACLE, 1988. Physicochemi-

cal and microbial stratification in a meromictic karstic lake of Spain. *Verh. Internat. Verein. Limnol.*, 23: 522-529.

VICENTE, E., M.A. RODRIGO, A. CAMACHO & M.R. MIRACLE, 1991. Phototrophic prokaryotes in a karstic sulphate lake. *Verh. Internat. Verein. Limnol.*, 24: 998-1004.

ZIMMERMANN, R. & L. A. MEYER-REIL, 1974. A new method for fluorescence staining of bacterial populations on membrane filters. *Kieler Meeresforsch.*, 30: 24-27.