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Factors Determining Annual Changes in Bacterial Photosynthetic Pigments in Holomictic Lake Cisó, Spain

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The pigments and biomass of anoxygenic phototrophic bacteria were measured during a year cycle in Lake Cisó (Girona, Spain). Two genera, *Chromatium* and *Chlorobium*, accounted for most of the bacterial population. The bacteria were present throughout the year despite complete mixing of the lake during fall and winter. This was possible because the sulfide production in the sediment was high enough to make the lake anaerobic to the very surface. Solar radiation, temperature, and biomass of *Chromatium* sp. were found to be important in determining pigment concentrations by correlation analysis. Sulfide concentration and biomass of *Chlorobium* spp. were found to be unimportant. A path analysis was performed to determine what percentage of the variability of pigments could be explained by the variables studied. Since a high percentage could be explained, it was possible to conclude that solar radiation, temperature, and biomass of *Chromatium* sp. were the main variables.

Waters colored red or green from photosynthetic bacterial blooms have been found in several lakes (3, 7, 11, 19, 25, 28). Their species composition and productivity have also been estimated in different environments (8, 20, 27), but no attempt has been made at quantifying photosynthetic pigments and to find which environmental factors determine the abundance of such pigments throughout the year. Lake Cisó offered the advantage of being the only described holomictic water body in the world where photosynthetic bacteria are present year round, thus offering a natural habitat where the bacteria are subjected to very different combinations of environmental conditions at different times of the year.

However, simultaneous changes in several variables result in a complex system, where relationships may not be immediately obvious. In this context, there has been a considerable increase in the use of multivariate statistical methods in microbiology in the last few years (2, 5, 10, 16, 23, 26). This growing interest is due to the realization by microbiologists of the fact that, with the appropriate techniques, much valuable information can be obtained from complex sets of data. As part of a study of the phototrophic bacteria in Lake Cisó, a small eutrophic, anaerobic, sulfureous, holomictic lake next to Lake Banyoles in northeastern Spain (13), we analyzed the relationships among photosynthetic pigments throughout the year, and we tried to establish which environmental and biological parameters were important in determining pigment concentrations in the lake. The results on factors affecting biomass changes will be reported elsewhere.

MATERIALS AND METHODS

Lake Cisó. The general physicochemical and biological characteristics of Lake Cisó have been described by Guerrero and Abella (13). The lake is a small anaerobic sulfureous water body of 457-m^2 surface area and 11-m maximum depth, located 300 m west of Lake Banyoles (Girona, Spain). It is fed by seepage of water rich in sulfate. This sulfate is reduced to sulfide in the anaerobic sediments, and sulfide diffuses through the whole water column in the winter and throughout the hypolimnion in the summer. Photosynthetic bacteria are present year round, but their species composition and abundance change substantially with the seasons (1, 14).

Chemical analyses. Sampling was done biweekly during 1978 with a 1.8-liter Ruttner bottle. Sulfide was determined by the methylene blue colorimetric method of Strickland and Parsons (Fisheries Research Board of Canada bulletin no. 167, Ottawa). Temperature was measured with a thermoresistor (NTC, Miniwatt). Pigments were extracted in 90% acetone, and absorption spectra were obtained with a Pye Unicam SP 1700 spectrophotometer. Bacteriochlorophyll concentration was calculated by the method of Takahashi and Ichimura (27). Carotenoids were transformed to arbitrary units per liter as previously described (14). Biomass was determined by direct counts of preparations stained with acid fuchsin and methylene blue on Sartorius membrane filters (0.45-μm pore diameter) (1), and cell volumes were determined from

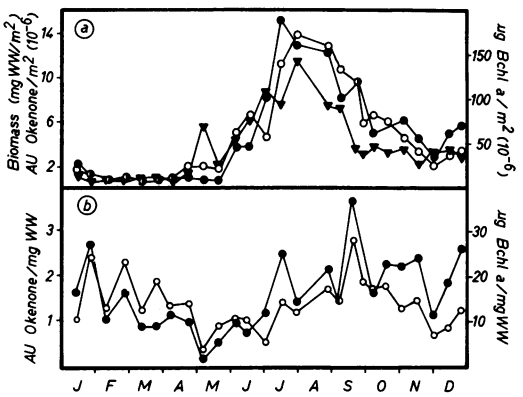

FIG. 1. Annual changes in integrated values of biomass, pigments, and specific pigment content of *Chromatium* sp. in Lake Cisó. (a) Biomass of *Chromatium* sp. (▼), Bchl *a* (○), and okenone (●) during 1978. Values have to be multiplied times 10^6 to obtain values per square meter. (b) Specific content of Bchl *a* (○) and okenone (●) in *Chromatium* sp. AU, Arbitrary unit; WW, wet weight.

scanning electron micrographs (I. Esteve, Ph.D. thesis, Universidad Autónoma de Barcelona).

Solar radiation. Direct solar radiation was not available for the period of the present study. However, the number of hours of sunshine without clouds was known from the meteorological station at the Girona-Costa Brava airport, only 25 km south of the Lake Cisó area and within the same river basin. Knowing solar radiation outside the atmosphere at 42° N latitude, a minimum of solar radiation could be calculated assuming that no light reached the lake when it was cloudy. Since annual changes of radiation calculated in this way will follow the same pattern as real radiation and since radiation was only used for correlation with other variables and not for absolute values of energy, this underestimate was deemed sufficient for the present study. Moreover, comparison of our results with radiation measured with a solarimeter during 1970 through 1971 (22) showed no significant differences in monthly averages with a paired samples Wilcoxon test, thus indicating a fairly constant annual cycle of solar radiation in the area.

Rainfall and evaporation. Daily rainfall was obtained from two National Meteorological Service weather stations at Castellfollit de la Roca (Girona) and Girona, both near Lake Cisó. Rainfall was incorporated into the last model as cumulative rain during the 2-month period before each sampling date. It was not included in the discussion of correlations because its influence was on biomass and not on pigments. Potential evaporation was calculated with Thornthwaite's equation (4).

Statistics. All the analyses were performed with the *Statistical Package for the Social Sciences* (18) in the Digital VAX-11/780 at the Computing Center of the Autonomous University of Barcelona. Normality of variables was tested with one-sample Kolmogorov-Smirnov tests (24). When variables included single values for each depth and sampling date, many of them were not normally distributed. None of the attempted transformations normalized these variables. When values from the whole water column were integrated for each sampling date, all of the variables were normally distributed, and Pearson correlation coefficients could be calculated. Multiple regression, partial correlation, and path analysis were performed with this last set of data.

RESULTS

Pigments in Lake Cisó. (i) *Chromatium*. Annual variations in pigment abundance and specific pigment content of *Chromatium* sp. are shown in Fig. 1. This genus was represented in Lake Cisó by one species, *C. minus* (1). Its pigments were the carotenoid okenone and bacteriochlorophyll (Bchl) *a*. In Fig. 1a the biomass of the microorganism is also shown. The biomass of *Chromatium* sp. was low during the winter; it started to increase in the spring and reached a maximum in the summer. Both carotenoids and Bchls presented the same annual cycle. Specific pigment contents (Fig. 1b) changed only slightly in *Chromatium* sp., from 4 to 40 µg of Bchl *a* per mg (wet weight) and from 0.3 to 3 arbitrary units of okenone per mg (wet weight). The biomass changed by 1 order of magnitude (Fig. 1a).

Typical summer and winter vertical profiles

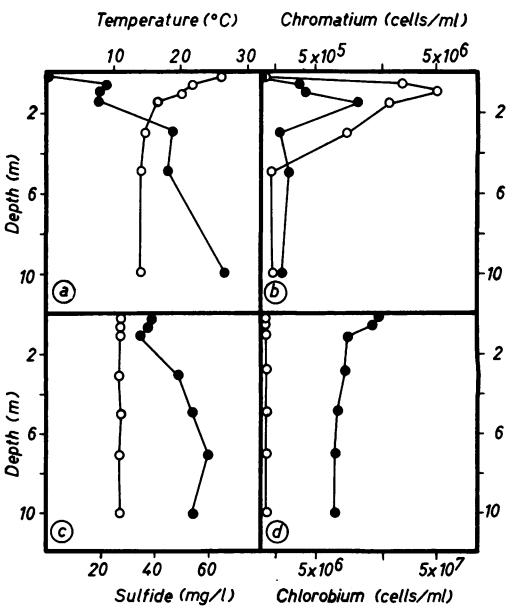

FIG. 2. Typical summer and winter vertical profiles in Lake Cisó. Temperature (○) and sulfide concentration (●) in the summer (a) and in the winter (c). Abundances of *Chromatium* sp. (○) and *Chlorobium* spp. (●) in the summer (b) and in the winter (d). Summer samples were taken on 2 July 1978, and winter samples were taken on 11 March 1978.

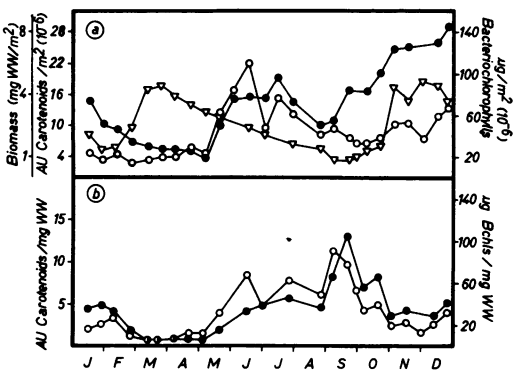

FIG. 3. Annual changes in integrated values of biomass, pigments, and pigment specific content of *Chlorobium* spp. (a) Biomass of *Chlorobium* spp. (▽), Bchls *c*, *d*, and *e* (○), and isorenieratene plus chlorobactene (●) during 1978. Values have to be multiplied times 10^6 to obtain values per square meter. (b) Specific content of Bchls *c*, *d*, and *e* (○) and isorenieratene plus chlorobactene (●) in *Chlorobium* spp. AU, Arbitrary unit; WW, wet weight.

are presented in Fig. 2. During the winter low numbers of *Chromatium* sp. were distributed uniformly throughout the water column. On the other hand, biomass in the summer was concentrated at the thermocline, where sulfide concentration was low and light intensity was high enough for the growth of the organism.

(ii) ***Chlorobium***. Figure 3 shows annual changes in pigments and biomass for *Chlorobium* spp. as well as specific pigment content (Fig. 3b). There were two groups of *Chlorobium* spp. in Lake Cisó: a brown species containing the carotenoid isorenieratene and Bchl *e* (*C. phaeobacteroides*) and a second group of at least two green varieties containing chlorobactene and Bchls *c* and *d* (17). For the present study, the two carotenoids and the three Bchls (Bchl *d* for brevity) were simply lumped together, since our purpose was to analyze relationships of *Chlorobium* spp. pigments versus those of *Chromatium* sp.

The biomass of *Chlorobium* spp. behaved almost completely opposite to that of *Chromatium* sp. It was low in January, but increased to a maximum in March and sustained large populations (10^7 cells ml^{-1}) until the summer, when it showed a decrease parallel to the increase in *Chromatium* sp. (Fig. 1a and 3a). After the collapse of the *Chromatium* sp. bloom, *Chlorobium* spp. attained another maximum in the fall.

Pigments had a different cycle. Carotenoids were low throughout the winter, increased to a maximum during the spring and summer, and decreased to an intermediate level during the fall. Bchl *d* was low during the winter, showed a low peak in the summer, and increased in the fall. This behavior was completely different from that of the *Chromatium* sp. pigments relative to the biomass of their species. *Chlorobium* spp. pigments did not follow changes in biomass.

Specific pigment contents were very low during the winter and spring, increased through the summer to a maximum in September, and decreased again toward the end of the year. Contents were between 1 and 120 μg of Bchl *d* per mg (wet weight) and between 1 and 15 arbitrary units of carotenoid per mg (wet weight), that is, a 10- to 100-fold difference, whereas *Chromatium* sp. pigments showed only a 10-fold variation.

In vertical profiles (Fig. 2), *Chlorobium* spp. were shown to exhibit uniformly high numbers (up to 4×10^7 cells ml^{-1}) during the winter and a stratified distribution in the summer, when it had a low maximum (2×10^6 cells ml^{-1}), just under the *Chromatium* sp. plate, and low numbers in the rest of the hypolimnion (Fig. 3b).

Correlation analysis. Pearson correlation coefficients among pigments and biomass of *Chromatium* sp. and *Chlorobium* spp. appear in Table 1. Partial correlation coefficients controlling for temperature, radiation, sulfide, and bio-

TABLE 1. Pearson correlation coefficients for integrated values of biomass and pigments of photosynthetic bacteria in Lake Cisó, 1978[a]

Organism	Variable	*Chromatium* sp.			*Chlorobium* spp.		
		Ok	Bchl *a*	Chr	Cc	Bchl *d*	Chl
Chromatium sp.	Ok	1.000	0.924[b]	0.748[b]	0.458[b]	NS	−0.605[b]
	Bchl *a*		1.000	0.721[b]	0.471[c]	0.444[d]	−0.545[c]
	Chr			1.000	0.523[c]	NS	NS
Chlorobium spp.	Cc				1.000	0.574[c]	NS
	Bchl *d*					1.000	NS
	Chl						1.000

[a] Only statistically significant coefficients are shown. Abbreviations: Ok, okenone; Chr, biomass of *Chromatium* sp.; Cc, isorenieratene plus chlorobactene; Chl, biomass of *Chlorobium* spp.; NS, not significant.
[b] $P < 0.001$.
[c] $0.01 > P > 0.001$.
[d] $0.05 > P > 0.01$.

TABLE 2. Partial correlation coefficients for relationships among *Chromatium* sp. and *Chlorobium* spp. parameters in Lake Cisó, 1978[a]

Variable pair	Control variable				
	Temp	Radiation	Sulfide	Chr	Chl
Ok-Bchl *a*	0.865[b]	0.852[b]	0.908[b]	0.837[b]	
Ok-Chr	0.815[b]	0.636[b]	0.815[b]	NA	0.750[b]
Bchl *a*-Chr	0.681[b]	0.584[b]	0.799[b]	NA	0.693[b]
Cc-Bchl *d*	0.631[b]	0.681[b]	0.593[c]	0.573[c]	0.574[c]
Cc-Chl	NS	NS	NS	NS	NA
Bchl *d*-Chl	NS	NS	NS	NS	NA
Cc-Ok	NS	NS	0.419[d]	NS	0.584[b]
Cc-Bchl *a*	NS	NS	0.433[d]	NS	0.570[c]
Cc-Chr	0.448[d]	0.409[d]	0.532[c]	NA	0.533[c]
Bchl *d*-Ok	0.505[c]	0.564[c]	NS	NS	NS
Bchl *d*-Bchl *a*	0.656[b]	0.739[b]	0.511[c]	0.464[d]	0.551[c]
Bchl *d*-Chr	NS	NS	NS	NA	NS
Chl-Ok	−0.471[d]	−0.677[b]	−0.593[b]	−0.727[b]	NA
Chl-Bchl *a*	−0.370[d]	−0.535[c]	−0.528[c]	−0.616[b]	NA
Chl-Chr	NS	NS	NS	NA	NA

[a] Abbreviations as in Table 1; NA, not applicable.
[b] $P < 0.001$.
[c] $0.01 > P > 0.001$.
[d] $0.05 > P > 0.01$.

masses of *Chromatium* sp. and *Chlorobium* spp. are presented in Table 2. For *Chromatium* sp. carotenoids, Bchl *a*, and biomass were all highly correlated among themselves. These correlations were not spurious, as demonstrated by partial correlation coefficients controlling for temperature, sulfide, radiation (Table 2), and combinations of these variables (data not shown).

Carotenoids of *Chlorobium* spp. and Bchl *d* were strongly correlated among themselves. On the other hand, none of the pigments showed a significant correlation with the biomass of the organism. These coefficients were not altered by controlling for temperature, radiation, sulfide, biomass of *Chlorobium* spp., or biomass of *Chromatium* sp. (Table 2), indicating that, whereas there was a tight coupling between the synthesis of carotenoids and Bchls, pigment abundances did not correspond to changes in biomass of *Chlorobium* spp.

Pearson correlation coefficients between bacterial and environmental parameters appear in Table 3. Partial correlation coefficients controlling for temperature, solar radiation, sulfide, and biomass of the organisms are presented in Table 4. Both okenone and Bchl *a* were highly correlated with temperature and with solar radiation. Biomass was also correlated with temperature and radiation. When partial coefficients were calculated, the correlations between Bchl *a* and biomass with temperature were shown to be spurious, but not that of okenone, whose coefficient was still high. This was a reflection of the high correlation between temperature and solar radiation, since the former is a direct consequence of the second. Thus, biomass and Bchl *a* seemed to depend strongly on light, whereas okenone depended on both light and temperature.

Concentrations of both pigments showed lack of correlation with sulfide, since the significant coefficients in Table 3 are shown to be spurious in Table 4, when controlling for temperature or radiation. This indicated that pigment synthesis was not dependent on sulfide concentration in the lake. Sulfide, however, could have a slight negative influence on the biomass of *Chroma-*

TABLE 3. Pearson correlation coefficients between parameters of *Chromatium* sp. and *Chlorobium* spp. and environmental variables in Lake Cisó, 1978[a]

Variable	Temp	Radiation	Sulfide
Ok	0.816[b]	0.775[b]	0.409[c]
Bchl *a*	0.688[b]	0.688[b]	0.443[c]
Chr	0.383[d]	0.522[b]	NS
Cc	0.353[c]	0.385[c]	NS
Bchl *d*	NS	NS	NS
Chl	−0.442[b]	−0.246[c]	NS

[a] Abbreviations as in Table 1.
[b] $P < 0.001$.
[c] $0.05 > P > 0.01$.
[d] $0.01 > P > 0.001$.

TABLE 4. Partial correlation coefficients for relationships between parameters of *Chromatium* sp. and *Chlorobium* spp. and environmental factors in Lake Cisó, 1978[a]

Variable pair	Control variable				
	Temp	Radiation	Sulfide	Chr	Chl
Ok-Temp	NA	0.510[b]	0.797[c]	0.863[c]	0.752[c]
Bchl *a*-Temp	NA	NS	0.636[b]	0.643[c]	0.574[b]
Chr-Temp	NA	NS	0.593[b]	NA	0.568[b]
Ok-Sulf	NS	NS	NA	0.606[c]	NS
Bchl *a*-Sulf	NS	NS	NA	0.629[c]	NS
Chr-Sulf	−0.320[d]	−0.357[b]	NA	NA	NS
Cc-Temp	NA	NS	NS	NS	0.433[d]
Bchl *d*-Temp	NA	NS	NS	NS	NS
Chl-Temp	NA	−0.428[c]	−0.411[d]	−0.413[b]	NA
Cc-Sulf	NS	NS	NA	NS	NS
Bchl *d*-Sulf	NS	NS	NA	NS	NS
Chl-Sulf	NS	NS	NA	NS	NA

[a] Abbreviations as in Tables 1 and 2.
[b] $0.01 > P > 0.001$.
[c] $P < 0.001$.
[d] $0.05 > P > 0.01$.

tium sp. This is probably due to the high sulfide concentrations in Lake Cisó, usually above upper limits for growth of *Chromatium* sp.

Bchl *d* was not correlated with any of them. Biomass of the organism presented a slight negative correlation with temperature and radiation. The correlation of carotenoids with temperature disappeared when controlling for radiation, showing it to be spurious (Table 4). Neither pigments nor biomass was correlated with sulfide. Controlling for temperature, radiation, or biomass did not alter this. Thus, pigments of *Chlorobium* spp. were not affected by either temperature or sulfide, although biomass did correlate negatively with temperature. Only carotenoids showed a very slight dependence on radiation.

Pearson correlation coefficients among pigments and biomass of the two genera are shown in Table 1. Carotenoids of *Chlorobium* spp. were correlated both with okenone and Bchl *a*, but these covariations were shown to be spurious when controlling for radiation, temperature, or biomass of *Chromatium* sp. The correlation of the *Chlorobium* spp. carotenoids with biomass of *Chromatium* sp., however, was real (Tables 1 and 2). Bchl *d* was correlated with neither biomass of the two genera nor okenone, but it was correlated with carotenoids of *Chlorobium* spp. and with Bchl *a*. When controlling for temperature or radiation Bchl *d* was shown to be also correlated with okenone. Thus, whereas carotenoids of *Chlorobium* spp. seemed to follow more closely *Chromatium* sp. biomass, Bchl *d* rather followed *Chromatium* sp. pigments. Biomass of *Chlorobium* spp. showed a negative correlation with pigments of *Chromatium* sp. and no correlation with its biomass.

Path analysis. Detalled analysis of correlations and partial correlations indicated which variables were important in determining pigment abundances in the lake, but correlations alone would not suffice to test whether the variables included in our study were all the important ones. Moreover, correlations would indicate neither the direction nor the strength of the relationships among variables.

The method of path analysis (9, 30, 31) was chosen to clarify this matter. In a first approximative model all of the variables considered important were included. This model had the problem that paradoxical relations appeared. For example, temperature turned out to have a negative influence, and radiation a negligible one, on *Chlorobium* spp. carotenoids (data not shown), whereas the correlation between these variables was positive (Table 3).

Elimination of some of the variables solved this problem, but it created a specification error. For example, temperature was excluded from a second model. Since temperature did have a real influence on okenone (Table 4), elimination of this variable resulted in lower explanatory power of the model. This was shown by the error terms and percentage of variance unexplained, shown in Table 5 for the three models mentioned. We used principal components analysis with Varimax rotation (18) to obtain a new set of independent variables which were uncorrelated with each other as a consequence of the nature of the method used (6). Then, multiple regressions were performed to obtain path coefficients from these new variables to pigments.

Figure 4 shows the space created by the three main factors or components (which together accounted for 81% of the variance in the data)

TABLE 5. Error terms and percent of variance not explained[a]

Variable	Model 1		Model 2		Model 3	
	ER	VNT	ER	VNT	ER	VNT
Bchl *a*	0.597	36	0.650	42	0.608	37
Ok	0.454	21	0.586	34	0.458	21
Bchl *d*	0.509	26	0.691	48	0.691	48
Cc	0.680	46	0.707	50	0.574	33

[a] ER, Error term; VNT, percentage of the variability not explained by variables in the model. Model 1, Model constructed including all the relevant variables. Model 2, Model constructed without temperature as an independent variable, as an example of an especification error. Model 3, Model constructed with the three first factors from a principal components analysis as independent variables (see the text for details).

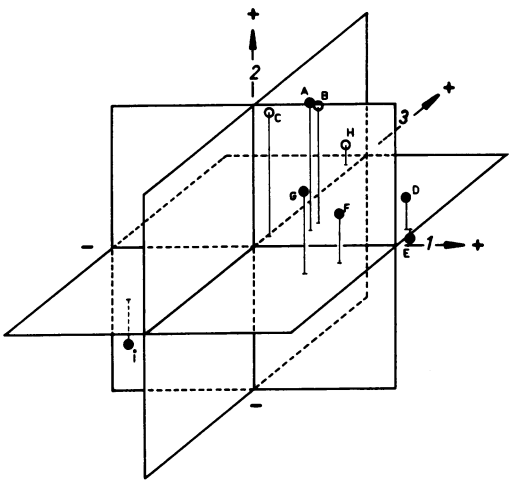

FIG. 4. Representation of the space created by the three first components from principal component analysis. Variables: A, temperature; B, solar radiation; C, evaporation; D, cumulative rainfall at Girona; E, rainfall minus evaporation; F, cumulative rainfall at Castellfollit; G, sulfide concentration; H, biomass of *Chromatium* sp.; I, biomass of *Chlorobium* spp. The coordinates for each variable are the respective loadings (equivalent to correlation coefficients) with each factor or component. The scales of the three axes are identical.

and the situation of the explanatory variables in such a space. From the distribution of the variables it could be easily seen that factor 1 corresponded to rainfall, factor 2 corresponded to solar radiation and related variables (temperature and evaporation), and factor 3 separated biomass of *Chromatium* sp. with a high positive value and biomass of *Chlorobium* spp. with a high negative value.

The model constructed with these factors as independent variables is shown in Fig. 5. Its error terms and percentage of each variable unexplained are presented in Table 5. In this analysis a satisfactorily high percentage of the variations of Bchl *a*, okenone, and *Chlorobium* spp. carotenoids could be explained, whereas the variations of *Chlorobium* spp. Bchls were explained to a lesser (but still high) degree. Factor 1 had low, negative influences, whereas factors 2 and 3 had medium to high positive influences on pigments. A rather interesting result was that sulfide and biomass of *Chlorobium* spp. could be excluded from the model without loss of explanatory power.

DISCUSSION

Path analysis was developed by Sewall Wright (30) as a method to test the assumptions of a particular model and to provide criteria by which to decide the best among several alternative models. These models have to assume linear, additive relationships among variables, where some variables are considered to be influenced by some independent variables (that is, there is causal order). The variability left unexplained after the influence of the independent variables has been considered is assumed to be due to unmeasured variables or to sampling error and is given by the error terms. Path analysis does not allow deduction of the causal order in a given system. This has to be proposed by the researcher on the basis of previous knowledge about the system. But the analysis permits testing of whether the assumptions about causal order are justified. We chose this method because, first, it allowed us to test whether all of the important variables had been included in our study by looking at the magnitude of the error terms. Second, we could determine quantitatively what percentage of the variability of each pigment was explained by our variables and in which way. Third, it permitted us to quantify the strength of each path, that is, which variables were more important for each pigment.

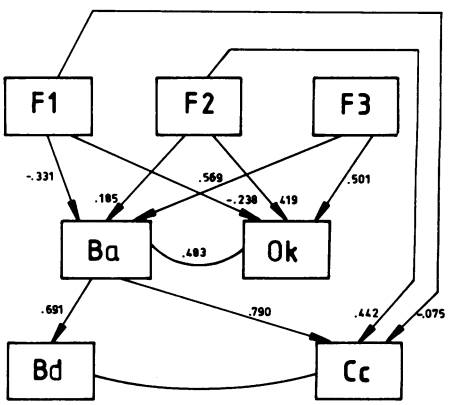

FIG. 5. Path diagram for the model derived from factors (F) from principal components analysis as independent variables and pigments as dependent variables. See Table 5 for error terms and percentage of variability not explained. In this graphic representation paths are represented by lines connecting the variables. Arrowheads indicate the direction of causality by pointing at the dependent variable. Path coefficients indicate the magnitude of the relationship. Thus, the positive influence of factor 3 on Bchl *a* (Ba) is greater than that of factor 2. The covariations between Bchls and the carotenoids of the same organism are shown with curved lines. This indicates an unanalyzed correlation, that is, nothing is stated about which is the independent variable. Pigments are probably related because of the common regulation of their synthesis by means of photosynthetic membrane synthesis. Ok, Okenone; Cc, isorenieratene plus chlorobactene. Bd, Bchl, *c*, *d*, and *e*.

Problems encountered in the construction of the first model were due to the well-known phenomenon of multicollinearity (15). Path coefficients are calculated by finding the standardized coefficients in a multiple regression in which a dependent variable is regressed on all the variables with paths leading to it (12, 29). When several of the independent variables in a regression are strongly correlated, such as is the case of radiation and temperature, multicollinearity appears. One way to circumvent this problem is to eliminate some of the variables. However, this results in an error of especification of the model (15), since some important variables are left out of the model and, thus, the amount of explained variations of the dependent variables diminishes. As an example, a model eliminating temperature was constructed. The error terms were larger, and the percentages of variation explained were lower, than for the complete model (Table 5).

We took advantage of the fact that the method for calculating the path coefficients is multiple regression of each dependent variable on all of the independent variables having some influence on them. Because of this, one of the possible solutions to the multicollinearity problem in normal multiple regression could be applied here. This is principal components analysis (6, 15, 23), in which the measured variables are reduced to a smaller set of orthogonal factors (that is, uncorrelated among them, thus solving the multicollinearity problem), which explains most of the variability in the data.

The resulting model (Fig. 5) showed several interesting features. Factor 1, related to rainfall and therefore to water flux through the lake, had a small negative influence on Bchl *a* and okenone, both strongly correlated with *Chromatium* sp. biomass. We interpret this as the result of Lake Cisó behaving as a chemostat, where sulfate-rich water enters at the bottom of the lake, and water with cells leaves the lake through the surface outflow. Higher rainfall implies a stronger flux through the lake. When this flux reaches a certain level *Chromatium* sp. is unable to reproduce fast enough to compensate and is washed out of the system, carrying its pigments away.

Factor 2, related to solar radiation and temperature, and factor 3, related to biomass, had a substantial positive influence on Bchl *a* and okenone. This is a result of *Chromatium* sp. being more abundant in the summer, when both radiation and temperature were high, and to the fact that pigments were strongly correlated with biomass.

Factor 3 had no significant influence on *Chlorobium* spp. carotenoids, but factor 2 had a very strong influence. Therefore, concentration of carotenoids was dependent on the concentration of *Chromatium* sp. pigments, radiation, and temperature, but not on the biomass of either bacterium. Synthesis of carotenoids in *Chlorobium* spp. was a response to the abundance of *Chromatium* sp. pigments above it and their shading effect.

Finally, Bchl *d* was the least satisfactorily explained variable (although 52% of its variability could be explained). It had a strong dependence on Bchl *a*, substantiating our conclusions about the shading effect of *Chromatium* sp. on *Chlorobium* spp. Its relations to the three factors were complicated by the simultaneous correlations with *Chromatium* sp. pigments. We did not attempt any solutions because they would have complicated the model unnecessarily. The lesser percentage of variance explained for Bchl *d* was probably due to the composite nature of the variable. In effect, brown and green species of *Chlorobium* competed in Lake Cisó, each one with its own pigments and requirements. These species lived in the lake at different times of the year (17), but were lumped together in the present study, and therefore important differences between them were ignored.

It is also of interest that biomass of *Chlorobium* spp. was not important in determining the abundance of pigments of this organism. *Chlorobium* spp. pigments were determined by the abundance of *Chromatium* sp. rather than by the abundance of *Chlorobium* spp.

Light and sulfide have been considered as the two most important factors affecting phototrophic bacteria in lakes (19–21, 27). Sulfur phototrophic bacteria are able to grow to the extent that sulfide is available to them. Thus, sulfide concentration would be expected to have an influence on biomass and, through it, on pigments. In our study, however, sulfide did not have any significant influence on pigments. This can be explained by the high concentration of sulfide found in Lake Cisó. Thus, variations in sulfide would not have an effect on bacteria as long as they would stay above limitation levels and below inhibitory levels.

Light quantity (solar radiation) was the main factor determining abundance of *Chromatium* sp. pigments and light quality (presence or absence of a selective filtering by *Chromatium* sp.), the main one affecting *Chlorobium* spp., as proposed by Abella et al. (1) and in agreement with Parkin and Brock (20). However, these last authors did not measure biomass, but only pigments. In our study biomass was not explained by light alone. Other variables will have to be analyzed to explain variations in the biomass of the organisms. Further experiments to measure the losses due to outflow, sedimentation, and predation are underway.

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