Summary and conclusions

Summary

The general objective of the thesis was to study the photosynthetic marker pigment signal in different lake compartments (water column, superficial sediment and sedimentary record) of Pyrenean high mountain lakes in order to evaluate the value of pigments as taxonomic markers and as indicators of the processes occurring at the different temporal scales associated to each of these processes. Some of the Pyrenean lakes have been intensively studied in terms of the seasonal variability of algal composition and phytoplankton production (Capblancq, 1972; Catalan, 1987; Catalan & Camarero, 1991; Catalan, 1991; Felip, 1997; Felip *et al.*, 1999a). In contrast, the algal group composition variability at regional scale has been poorly studied (Capblancq, 1972; Margalef *et al.*, 1975; Vilaseca, 1978). In order to see the local effect in the pigment signal of changes in factors such as irradiance or mixing regime, Lake Redon was sampled seasonally. In order to evaluate the regional variability, we sampled 80 Pyrenean high mountain lakes covering a wide range of lake areas, bedrock type and catchment vegetation giving them different morphometric and physico-chemical characteristics. Finally, in order to use indicator pigments as long term system changes indicators, the primary production of Lake Redon was reconstructed along the last ten thousand years.

Methodological aspects

A catalog was created with the information of the absorption spectra and retention time of pigments belonging different algal groups including diatoms, chlorophytes, chryptophytes, benthic and planktonic cyanobacteria, dinoflagellates, chlorobiaceae, chromatiaceae, mosses, macrophytes and planktonic crustacea. Samples were obtained from algal cultures and field samples collected from several Pyrenean lakes.

A chromatographic analytical protocol was defined for analyzing samples with a greater complexity in terms of peak number per chromatogram such as those found in sediment samples. Within these samples, the chromatogram peak integration and subsequent quantification was more reproducible when using solvents with a certain percentage of water such as, methanol 90% and acetone 90%, than when using dimethyl sulphoxide 100% or this solvent in combination with acetone. Acetone 90%, in combination with a disruptive technique such as ultrasounds, was the solvent with a higher extraction capacity.

There were no qualitative differences in terms of peak number and spectral characteristics when analyzing the extract from lyophilized or fresh sediment. On the other hand, the measured pigment concentration was reduced, on average, by 28% with the used method when lyophilizing the samples. The major inconvenient in the use of fresh material was the need of previously estimating the water content, which was necessary in order to avoid that the final extract would have more than 10% of water.

The results of using different columns on the chromatogram peak resolution, composition and quantification indicated that the two not end-capped columns (Spherisorb-ODS1 i YMC30) could differentiate several conflictive isomers such as lutein and zeaxanthin which were not resolved with the end-capped column (Nova-Pack C18). The use of YMC30 column presented as the main inconvenient that it was not able to adequately resolve canthaxanthin from chlorophyll b and β,β-carotene from pheophythin a1, and was unable to detect oscillaxanthin.

Study of the pigment signal at seasonal scale

In Lake Redon, algal group Chlorophyll a estimates and the algal biovolume had a significant correlation for all algal groups with the exception of chlorophytes during the ice-free period. In contrast, in some of the samples from the beginning of the ice-covered period there was a weaker relationship between both indicators than the ice-free period.

The ratios between algal group marker pigments and their respective biovolume were constant within the photic zone of the lake (> 1% surface irradiance) for most of the pigments and groups, including chlorophytes. The ratios had higher and more variable values in the samples situated below the photic zone or below the ice cover. The violaxanthin-chrysophyte biovolume ratio presented an opposed tendency to other pigment-biovolume ratios, which increased in inverse proportion to the depth of the sample.

The variability in the relationship between both estimates was probably due to changes in the cellular pigment content in response to photoacclimation and photoprotection and to changes in the species assemblages.

Study of the pigment signal at regional scale

The proportion of each algal group to the phytoplankton population was estimated from the relationship between a marker pigment and the chlorophyll a from the same group in a representative group of high mountain lakes from the Pyrenees. In relative terms, chrysophytes were the dominant group in the deep chlorophyll maxima of the greatest number of the lakes, followed by cryptophytes and chlorophytes. Diatoms and cyanobacteria dominated in a reduced number of lakes, while dinoflagellates dominated in only one lake. The minimum combination of environmental variables explaining most of the algal group variability was composed by calcium, phosphorus, fish presence/absence and macrozooplankton abundance. Phosphorus concentration, fish presence/absence and

macrozooplankton abundance were positively correlated with the first axes of the redundancy analysis indicating the existence of a trophic gradient. Cryptophytes were associated to lakes with greater phosphorus concentrations and with fish present, while chrysophytes were more abundant at the other extreme of the gradient. Chlorophytes were correlated with high densities of macrozooplankton. The other algal groups, diatoms, cyanobacteria and dinoflagellates were independent of the trophic gradient. The second gradient was associated with the concentration of calcium. Cyanobacteria and diatoms were positively correlated with this gradient, while chloropytes and in a lower degree, chrysophytes, were negatively correlated. Cryptophytes and dinoflagellates were independent of the calcium gradient.

The planktonic algal group pigment indicators reaching the sediment were alloxanthin from cryptophytes and lutein from chlorophytes. None of the indicator pigments of chrysophytes and dinoflagellates were detected in the sediment despite being the former the most abundant planktonic algal group in many Pyrenean lakes. On the other hand, the benthic algal groups indicator pigments were zeaxanthin and myxoxanthophyll from cyanobacteria and diatoxanthin from diatoms. The results suggested that fucoxanthin signal in the sediment was mostly attributable to diatoms growing *in situ* in the biofilm of the superficial sediment. The signal of photosynthetic bacteria was also found in the sediment samples.

The most significant gradient described by the pigment composition ranged from a benthic to a planktonic signal. This gradient was a result of the wide range of light penetration within the surveyed lakes. The pigments that better represented this gradient were alloxanthin (planktonic signal) and diatoxanthin (benthic signal). In those lakes where less than 10% of the light reached the biofilm the ratio of these two pigments was correlated with lake maximum depth. Therefore, this ratio was a good indicator of the relative contribution to the overall primary production of these two environments. There were also other factors explaining the remaining variability of the pigment signal. pH was related with pigment preservation since it was the main factor explaining the variance of the chlorophyll a preservation index and the ratio of chlorophyll a feopigment derivatives to total carotenoids. The percentage of alpine meadows within the lake catchment was related to the pheophytin concentration in the sediment. Finally, the photosynthetic bacteria indicator pigments were mainly found in relatively shallow lakes with large afforested catchment areas probably due to the higher concentration of allochtonous organic matter.

Paleolimnological application

The variability in the production of Lake Redon along the Holocene was reconstructed using organic carbon, total phosphorus and a-phorbins (sum of chlorophyll a, pheophytin and pheophorbide). The production signal had two main discontinuities along the record which separated three different zones: from 10000 to 8200 y. BP, from 8200 to 1500

y. BP and from 1500 y. BP to present. From these three zones, the two situated at the two extremes had the highest production, especially those ranging from 1500 y. BP to present.

The period from 10000 to 8200 y BP was characterised by a predominance of the diatom and cyanobacterial pigments, which indicated a predominance of the benthic communities in the lake during this period. The good preservation of chlorophyll a together with the presence of very labile indicator pigments suggested that there was an *in situ* growth of these communities, either growing in the same depositional area or very close to it. The most likely hypotheses explaining such scenario was the existence of a lower water level in the lake as a result of the characteristic climate of the period (high temperatures in summer, low temperatures in winter and reduced precipitation). The high production of the period was attributed to the higher external load of phosphorus incoming from the catchment due to the poorly vegetated conditions during the early soil development. On the other hand, oscillations of the production signal were attributed to changes in the internal load of phosphorus due to changes in the ice cover length.

The period from 8200 to 1500 y. BP was more stable in terms of production. It was so low that there was hardly any signal of indicator pigments. Only the less labile pigments were detected (alloxanthin, astaxanthin and canthaxanthin). The decrease in the lake production during this period was attributed to a reduction of the external phosphorus loading as a result of an increase of the vegetation in the catchment, and also to lower ice-cover length that resulted in a reduction of the internal phosphorus loading. The variability of both factors was attributed to changes in the climatic conditions.

From 1500 y. BP to present there was a marked increase in the fluxes of all the productivity indicators. The groups of primary producers that mostly contributed to the productivity signal were planktonic algal pigment indicators. Photosynthetic bacterial pigments were detected for the first time in the history of the lake during this period. This sudden change in the production and in the primary production community composition was associated with an increase in the sedimentation rates up to four times higher than the previous period. It was related with an increase of the external nutrient loads due to soil perturbations in the catchment. The onset of sheep grazing from this period could represent a sufficient perturbation explaining an increase in soil erosion and a subsequent fertilisation of the lake.

Conclusions

1- The analytical protocol that gave the most satisfactory results for pigment analysis in sediment samples was based in the use of acetone 90% in combination with a disruptive technique such as ultrasounds for extracting the pigments from a sample previously lyophilised and the use of a chromatographic system based in the use of the mobile phase described by Kraay *et al.* (1992) with a non end-capped C18 column.

2- In very oligotrophic lakes such as Lake Redon, algal group chlorophyll a estimated with CHEMTAX was closely related with cellular biovolume of chrysophytes, dinoflagellates and cryptophytes during the ice-free period. In contrast, the method can erroneously estimate the chlorophyte cellular biovolume when the concentrations are low and the species are colonial.

3- Below the ice and very likely under any low irradiance conditions and with sufficient nutrients, the estimated chlorophyll a with CHEMTAX may overestimate the algal biovolume if the ice-free period relationship is applied.

4- In Lake Redon, the ratios between an algal group marker pigment and the biovolume of its respective algal group were constant within the photic zone of the lake (> 1% surface irradiance) for most of the pigments and algal groups.

5- The violaxanthin-chrysophyte biovolume ratio presented an opposed tendency to other pigment-biovolume ratios, which increased in inverse proportion to the depth of the sample. This result could indicate the use of violaxanthin by chrysophytes as a photoprotective pigment.

6- Within the representative set of Pyrenean high mountain lakes, chrysophytes were the algal group that dominated the deep chlorophyll maxima in a greater number of lakes, followed by criptophytes and chlorophytes. The other groups (dinoflagellates, diatoms and cyanobacteria) contributed to a lesser extent to the phytoplankton of the deep chlorophyll maxima.

7- The first gradient in the variability of algal group composition along the Pyrenees was defined by the lake's trophic state. Criptophytes were associated with higher phosphorus lakes that also had fish present, while chrysophytes dominated at the

other extreme of the trophic gradient. Chlorophytes were correlated with higher macrozooplankton abundance.

8- The second gradient in the variability in the algal group composition was correlated with calcium concentration. Cyanobacteria and diatoms were positively correlated with this gradient, while chlorophytes and to a lower degree chrysophytes were negatively correlated.

9- Within the sediment, the planktonic algal group indicators were alloxanthin from cryptophytes and lutein from chlorophytes. There was no indicator pigment of chrysophytes or of dinoflagellates that persisted in the sediment despite being the former the dominating group in many Pyrenean high mountain lake phytoplanktonic communities.

10- The benthic algal group indicator pigments found in the sediments were zeaxanthin and myxoxanthophyll from cyanobacteria and diatoxanthin from diatoms.

11- The most significant gradient described by the pigment composition ranged from a benthic signal in those lakes where light was reaching the bottom to a planktonic signal in deep lakes where the photic zone did not reach the bottom.

12- In those lakes where less than 10% of the light reached the biofilm the ratio of these two pigments was correlated with lake maximum depth. Therefore, this ratio was a good indicator of the relative contribution to the overall primary production of these two environments.

13- pH was the factor that had the highest influence in chlorophyll a preservation. It explained the greatest part of the variability of the chlorophyll a preservation index and of the ratio between chlorophyll a derived feopigments and total carotenoids. Catchment vegetation characteristics were the second factor affecting these two ratios.

14- Photosynthetic bacteria indicator pigments were mainly found in relatively shallow lakes with large afforested catchment areas probably due to the higher concentration of allochtonous organic matter.

15- Reconstruction of the variability in the production of Lake Redon along the Holocene showed three clearly different zones. A first period with a high production (from 10000 to 8200 y. BP), followed by a period of low production during mid Holocene (8200 to 1500 y. BP) and the last period (from 1500 y. BP up to present) with the highest production values of the whole Holocene.

16- The origin of the production signal at the early Holocene had a predominantly benthic origin, while during the last 1500 years it was mainly of planktonic origin.

17- Until the end of the mid Holocene period the variability in the Lake Redon primary production was attributed to the changes in the internal and external phosphorus loads associated with the climate changes, which took place during this period. Since 1500 y. BP, the variability was mainly attributed to changes in the external phosphorus loads related with soil perturbations after the onset of sheep grazing activity.