

## Stratigraphy of specific algal and bacterial carotenoids in the sediments of Lake Varese (N. Italy)

Piero Guilizzoni, Andrea Lami, Delio Ruggiu & Giuliano Bonomi  
C.N.R. Istituto Italiano di Idrobiologia, Largo V. Tonolli 50/52 – 28048 – Pallanza, Italy

Keywords: paleolimnology, sedimentary carotenoids, diatoms

### Abstract

A study on the succession of some phytoplankton populations in the very eutrophic Lake Varese over the past 80–100 years, was based on thin-layer chromatography of four group-specific carotenoids (echinenone, lutein, myxoxanthophyll and oscillaxanthin). The development of diatoms was traced by microscope counts of their frustules. In addition, the carotenoid okenone, peculiar to some *Chromatium* species, was identified and related to lake trophic conditions (the *Chromatium* species are obligate anaerobic bacteria, and use H<sub>2</sub>S).

### Introduction

During the past 2–3 decades, a great number of paleolimnological studies have dealt with sedimentary chlorophylls and total carotenoids (Guilizzoni *et al.*, 1982; Swain, 1985). There are, however, relatively few studies on the occurrence and distribution of specific algal carotenoids in lake sediments (Züllig, 1982). Among these pigments, the carotenoids of various blue-green algae have been studied extensively, this because Cyanophyta occupy a central position in the eutrophication problem of inland waters. For example, it is known that the pigment oscillaxanthin is largely restricted to some species of the blue-green alga *Oscillatoria* and is particularly abundant in *O. rubescens*. Thus, the concentration of this xanthophyll in the core profile can be useful in detecting the past development of this taxonomic group (Griffiths, 1978; Engstrom *et al.*, 1985).

In addition to plant pigments, those of photosynthetic bacteria have been successfully separated from their original population and used as valuable paleoecological indicators (Brown *et al.*, 1984; Züllig, 1984 and 1985).

Although several problems still remain partially

unresolved (e.g. diagenesis, preservation/degradation), no doubt exists about the usefulness of this approach, particularly when the pigment data are related to concurrent investigations that include other chemical and biological parameters.

In the study presented here, along the lines followed by Züllig (1981, 1982) and Brown *et al.* (1984), we have attempted to trace the development over the last century of some phytoplanktonic groups, and of photosynthetic purple sulfur bacteria (*Chromatium* spp.) in the subalpine hypertrophic Lake Varese (N. Italy). This was accomplished by analysis of group specific carotenoids in a sediment core. The study was supplemented by counting frustules of diatoms from the Centricae and Pennatae. Since all these organisms are strongly affected by the process of eutrophication, their changes with time will reflect the trophic status of the waters.

### Study area and methods

Lake Varese is a very eutrophic, relatively small (15 km<sup>2</sup>) well buffered (total alkalinity: 2–3 meq·l<sup>-1</sup>) water body, with maximum depth

of 26 m ( $\bar{z}=11$  m). The lake is generally stratified from May to November, during which time the hypolimnion undergoes a strong deoxygenation. Spectacular *Microcystis aeruginosa* blooms commonly take place in the superficial waters.

A 60-cm sediment core was collected on September 1983 from the deepest area of the lake.

Thin layer chromatography of carotenoids was performed on 1.5 cm sediment sections in the first 9 cm of the core, every 3 cm from 9 to 15 cm, every 5 cm from 15 to 30 cm and every 10 cm in the remaining part. The pigments were extracted and their amount (mg) calculated according to Züllig (1982); the concentration was expressed as percent of carotenoids per g organic weight. Carotenoids from pure strains of algae (NIVA's culture collection, Blindern, Norway) were used as reference standards.

Sample preparation for microscope counts of diatom frustules was based on Battarbee's method (1973) with minor modifications (Candido *et al.*, in press).

## Results and discussion

Echinenone, myxoxanthophyll and oscillaxanthin were studied as characteristic carotenoids of blue-green algae; lutein and okenone as distinctive pigments of green plants and of some purple sulfur bacteria of the genus *Chromatium*, (Fig. 1). The more ubiquitous  $\beta$ -carotene and the pool of total carotenoids were ascertained in order to obtain a general picture of the evolution of the whole phytoplankton community.

The sediments of Lake Varese show clear laminae only for the first 15–20 cm, where the percent of organic matter varies from the basic value of 11% to 21% dry wt. (Lami, 1985). Calcium carbonate concentration is high throughout (ca. 20% dry wt. in the lowest part of the core) but increases markedly during the last 15–20 years (ca. 45% in the surface; Lami, 1985). Such changes are interpreted as a product of biogenic origin (Adams *et al.*, 1978).

In spite of the available literature on sedimentation rates in Lake Varese, calculated as  $1.3 \text{ cm} \cdot \text{yr}^{-1}$

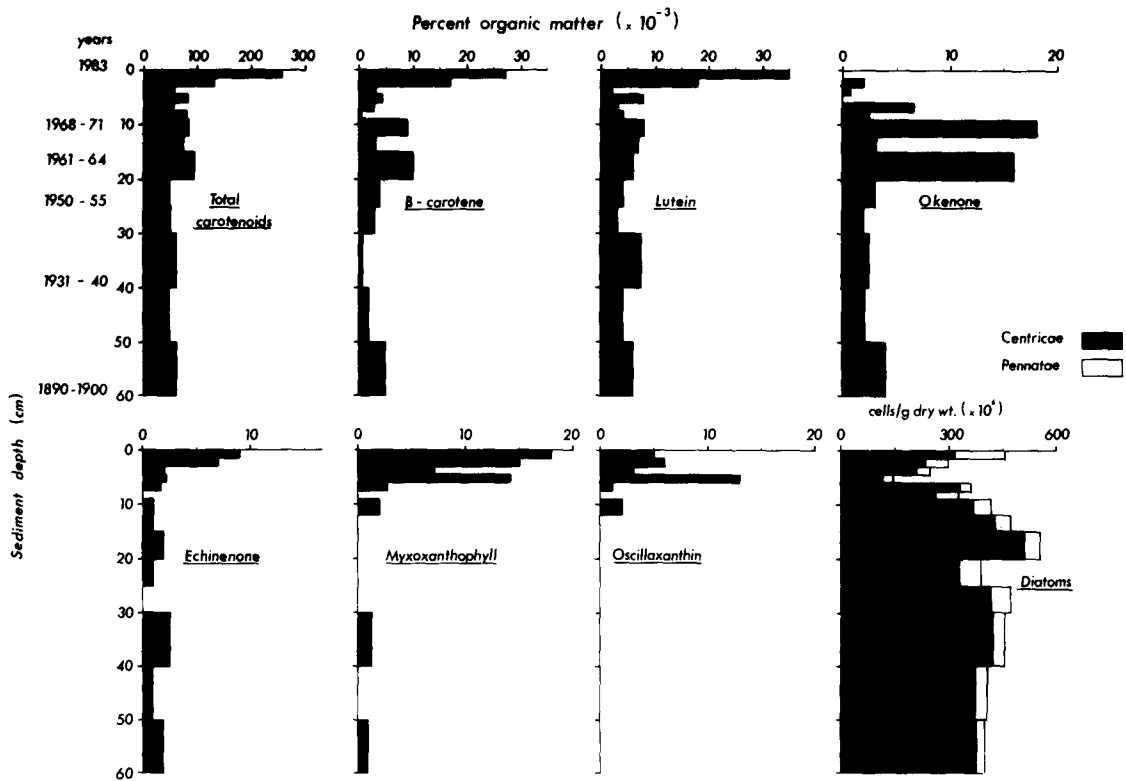


Fig. 1. Carotenoid stratigraphy of the sediments of Lake Varese. Depth distribution of diatoms is also shown.

by the  $^{137}\text{Cs}$  method (Ravera & Premazzi, 1971), the pigment and diatom data cannot be expressed as mg or number of cells sedimented per year, but only as percentages on an organic and dry weight basis respectively (Fig. 1). This was done because we do not know a mean sedimentation rate (although surely lower than the present) for the period prior to the 1960's. Approximate core dating (Fig. 1) was estimated by considering the effect of vertical compaction (Håkanson & Källstrom, 1978) on about  $2\text{ cm}\cdot\text{yr}^{-1}$  of wet material recently deposited (Lami, 1985).

The total carotenoids,  $\beta$ -carotene and lutein profiles are very similar with maximum values in the superficial layers (Fig. 1). Oscillaxanthin was detected in appreciable quantities from 9–12 cm upwards and its values parallel those of myxoxanthophyll. The appearance of oscillaxanthin is indicative of the onset of blooms of *Oscillatoria* in the lake that began in the late 1960's. The presence of *O. rubescens*, however, was noted by Tonolli & Bonomi (1965) as early as 1953. The apparent discrepancy with the core analysis is probably due to (i) the low density of the species in the 1950's (ca.  $30\times 10^6\text{ cells}\cdot\text{m}^{-3}$ ), which in turn results in quantities of pigment too small to be easily measured chromatographically, and (ii), to the patchy distribution of the carotenoids in different areas of the lake facilitated by the filamentous nature of *Oscillatoria* which might cause it to be easily moved about by currents. Judging from the oscillaxanthin content, this blue-green alga reached its highest population density about 1975 (4.5–6 cm layer) and then decreased in more recent times. The reduction probably corresponds to an increase in different species of Cyanophytes evidenced by increases in myxoxanthophyll and echinenone (Fig. 1). Shifts in the ratio oscillaxanthin/myxoxanthophyll provide further evidence for increased species variability in the last 15 years or so. These data are consistent with the field phytoplankton results (Ruggiu *et al.*, 1981) and with the current annual inspections that reveal continuous and heavy blooms of *Microcystis aeruginosa* from the beginning of summer to December. It is important to note that echinenone and myxoxanthophyll distributions in the sediment core indicate the quite abundant concentrations of these organisms during the last century.

Lutein, the main pigment of Chlorophyceae has

tripled its basic concentration of ca.  $5\times 10^{-3}\%$  organic matter in recent years, reaching with  $\beta$ -carotene the highest concentration (ca.  $25-30\times 10^{-3}\%$  o.m.). The most important species in the class is *Sphaerocystis schröteri*. In general, green algae (together with diatoms) are the most important group in spring and at the end of winter.

During this century the diatom populations also show a very high cell density per g dry wt. (Fig. 1): these values are 3–4 times higher than in Lake Mergozzo, a small, moderately productive lake of low alkalinity situated in the same geographic area as Lake Varese (Candido *et al.*, in press). A sharp reduction of the cell density of the Centricae, the dominant group in Lake Varese, occurs between 7 and 15 cm (about 1965–1975: Fig. 1). It seems likely that the massive development of blue-green algae displaced the diatoms in that period.

*Stephanodiscus hantzschii*, and to a lesser extent *Melosira granulata*, both well-known species of eutrophic lakes (Margalef, 1983), occur with the unexpected *Cyclotella* spp. the dominant form in Lake Varese (Lami, 1985). This may reflect frustule dissolution in hard water (Brugam, 1983). The A/C (araphidineae/centrales) ratio is always below 1 and therefore not in agreement with the classification proposed by Stockner (1971), and the Centricae species are always dominant over those belonging to the Pennatae group. These features are discussed in a forthcoming paper (Lami *et al.*, in press). The phytoplankton community, as a whole, increased dramatically from about the mid-1970's. The late 1950's was the time when the lake became very eutrophic. Bonomi (1962a), for instance, comparing the profundal benthos described for the period 1956–1957 with that studied again in 1962 (Bonomi, 1962b), demonstrated that *Chironomus anthracinus* had disappeared from the profundal zone. He attributed this catastrophic change to the extreme oxygen deficits that were developing during late winter, the pupation-period of *C. anthracinus* in Lake Varese (Bonomi, 1964 and 1969). Recent paleolimnological data (Bonomi, unpublished) confirm that *Chironomus* head capsules are present up to the 12 cm depth in the core but are completely missing in the more recent sediments. The development of greater anaerobiosis is also supported by some additional chemical and biological investigations (Lami, 1985) and by the okenone stratigraphy (Fig. 1). The high concentration of this

*Chromatium* carotenoid in all deep sediment core sections indicates an anaerobic hypolimnetic condition for the entire period represented by the core (Züllig, 1984). From 15–20 cm level (1960's) up to the superficial layer, the okenone concentration showed wide variation with near zero values in some sections and very high concentrations in others. The highest concentrations exceeded four times the pre-1960 values. Parking & Brock (1980) and Brown *et al.* (1984) have both noted the disappearance of photosynthetic bacteria when light is limiting. The heavy algal blooms of L. Varese in recent years may have reduced the light penetration so completely as to cause population crashes of *Chromatium*.

Both total and individual carotenoid concentrations measured in Lake Varese are in general higher than those reported by Züllig (1982) for some eutrophic Swiss lakes. However, Lake Varese is in one of the most populated and industrialized areas in Italy.

On the other hand, from the present study it appears that Lake Varese is a lake 'naturally productive' as suggested by its morphometric characteristics (low mean depth, large shallow area, etc.) and by the geology of its drainage basin.

In this context, the phytoplankton production of  $160 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-2}$ , estimated by Guilizzoni *et al.* (1983) for a period prior to 50–70 years ago, is well in accordance with the conclusions of this work. The further increase in production occurring in more recent years must be viewed as the result of intensified human activities acting upon a naturally productive lake.

### Acknowledgements

We want to express our gratitude to Dr. Hans Züllig (Rheineck-Switzerland) for his assistance in the TLC carotenoids analysis. The critical reading of the text by Dr. J. E. Sanger (Dept. of Botany-Bacteriology, Ohio Wesleyan University, Delaware, U.S.A.), Prof. M. S. Adams (Dept. of Botany, University of Wisconsin, Madison, U.S.A.) and Dr. H. Züllig is fully acknowledged.

### References

- Adams, M. S., P. Guilizzoni & S. Adams, 1978. Sedimentary pigments and recent primary productivity in Northern Italian lakes. *Mem. Ist. ital. Idrobiol.* 36: 267–285.
- Battarbee, R. W., 1973. A new method for the estimation of absolute microfossil numbers, with reference especially to diatoms. *Limnol. Oceanogr.* 18: 647–653.
- Bonomi, G., 1962a. La dinamica produttiva delle principali popolazioni macrobentoniche del Lago di Varese. *Mem. Ist. ital. Idrobiol.* 15: 207–254.
- Bonomi, G., 1962b. Le grandi modificazioni nella fauna macrobentonica del Lago di Varese intervenute nel periodo 1957–1962. *Acqua Industriale.* 4: 1–4.
- Bonomi, G., 1964. Un nuovo aspetto dell'evoluzione del Lago di Varese: la comparsa di situazioni meromittiche. *Mem. Ist. ital. Idrobiol.* 17: 231–246.
- Bonomi, G., 1969. Una ulteriore fase di declino del Lago di Varese. *Naturae Montagna* 9: 17–22.
- Brown, S. R., H. J. McIntosh & J. P. Smal, 1984. Recent paleolimnology of a meromictic lake: fossil pigments of photosynthetic bacteria. *Ver. int. Ver. Limnol.* 22: 1357–1360.
- Brugam, R. B., 1983. The relationship between fossil diatom assemblages and limnological conditions. *Hydrobiologia* 98: 223–235.
- Candido, A., P. Panzani & D. Ruggiu, (in press). Diatom remains in sediment core 1 m long from the subalpine Lake Mergozzo (Italy). *Mem. Ist. ital. Idrobiol.* 41.
- Engstrom, D. R., E. B. Swain & J. C. Kingston, 1985. A paleolimnological record of human disturbance from Harvey's Lake, Vermont: geochemistry, pigments and diatoms. *Freshwat. Biol.* 15: 261–288.
- Griffiths, M., 1978. Specific blue-green algal carotenoids in sediments of Eastwaite water. *Limnol. Oceanogr.* 23: 777–784.
- Guilizzoni, P., G. Bonomi, G. Galanti & D. Ruggiu, 1982. Basic trophic status and recent development of some Italian lakes as revealed by plant pigments and other chemical components in sediment cores. *Mem. Ist. ital. Idrobiol.* 40: 79–98.
- Guilizzoni, P., G. Bonomi, G. Galanti & D. Ruggiu, 1983. Relationship between sedimentary pigments and primary production: evidence from core analyses of twelve Italian lakes. *Hydrobiologia* 103: 103–106.
- Håkanson, L. & A. Källström, 1978. An equation of state for biologically active lake sediments and its implications for interpretations of sediment data. *Sedimentology* 25: 205–226.
- Lami, A., 1985. Il Lago di Varese: ricostruzione della sua recente evoluzione trofica attraverso uno studio paleolimnologico. Tesi di laurea in Scienze Biologiche, Univ. di Milano: 192 pp.
- Lami, A., D. Ruggiu, P. Guilizzoni & A. Panzani, (in press). Subfossil diatom remains in a short sediment core of the eutrophic Lake Varese, Italy. *Mem. Ist. ital. Idrobiol.*, 42.
- Parkin, T. B. & T. O. Brock, 1980. Photosynthetic bacterial production in lakes: the effects of light intensity. *Limnol. Oceanogr.* 25: 711–718.
- Ravera, O. & G. Premazzi, 1971. A new method to study the history of any persistent pollution in a lake by the concentration of  $^{137}\text{Cs}$  from fall-out. *Proceedings of the Intern. Symp. on 'Radioecology applied to the protection of man and his environment'*, Rome 7–10 September 1971 Commission of the European Communities 'u' 697 D.G.3: 703–719.

- Ruggiu, D., C. Saraceni & R. Mosello, 1981. Fitoplancton, produzione primaria e caratteristiche chimiche di un lago fortemente eutrofizzato: il Lago di Varese. *Mem. Ist. ital. Idrobiol.*, 39: 47–64.
- Stockner, J. G., 1971. Preliminary characterization of lakes in the experimental lakes area, North-western Ontario, using diatom occurrence in sediments. *J. Fish. Res. Bd Can.* 28: 265–275.
- Tonolli, V. & G. Bonomi, 1965. Stato biologico del Lago di Varese nelle situazioni passata e presente. In *Amm. Prov. Varese (ed.), L'inquinamento del lago di Varese, indagini-rilevamenti-proposte*: 89–108.
- Züllig, H., 1981. On the use of carotenoid stratigraphy in lake sediments for detecting past developments of phytoplankton. *Limnol. Oceanogr.* 26: 970–976.
- Züllig, H., 1982. Untersuchungen über die Stratigraphie von Carotinoiden im geschichteten Sediment von 10 Schweizer Seen zur Erkundung früherer Phytoplankton-Entfaltungen. *Schweiz. Z. Hydrol.* 44: 1–98.
- Züllig, H., 1984. Vorläufige Mitteilungen über das Vorkommen des aus Purpurbakterien stammenden Pigmentes Okenon in Sedimenten. *Schweiz. Z. Hydrol.* 46: 297–300.
- Züllig, H., 1985. Carotenoids from plankton and photosynthetic bacteria in sediments as indicators of trophic changes: evidence from the late glacial and the early Holocene of Lobsigensee. In G. Lang (Ed.), *Swiss lake and mire environments during the last 15000 years*. J. Cramer, Vaduz: 143–147.

Accepted 18 March 1986.