Aquatic Sciences 54, 3/4, 1992

1015-1621/92/040321-10 $ 1.50 + 0.20/0

Role of pigments on algal communities and photosynthesis

A. Lami [1], P. Guilizzoni [1], D. Ruggiu [1], B. Polli [2], M. Simona [2], A. Barbieri [2]

[1] CNR-Istituto Italiano di Idrobiologia, Verbania-Pallanza (Italy)
[2] Laboratorio Studi Ambientali, Sezione Protezione Acque, Dipartimento Ambiente, Lugano (Switzerland)

Key words: Algal pigments, algal communities, photosynthesis, Lake Lugano (Lago di Lugano).

ABSTRACT

A one-year study of phytoplankton, primary production and related physical and chemical factors was made in a Swiss basin of Lake Lugano (Lago di Lugano). The chlorophylls and 12 carotenoids were analyzed with a TLC technique. The carotenoid monitoring was considered to be particularly interesting, because the role of these pigments in freshwater algae is still very poorly documented by field studies. The dependence of photosynthesis on several factors was statistically evaluated. Evidence was found of light-adaptation phenomena. The variations of photosynthetic activity and efficiency largely depended on the light regime in the few days before the field observations and on the cellular content of chlorophylls and single carotenoids, whose concentrations in their turn were closely linked with light, temperature, average cell size, and with the actual species assemblage.

1. Introduction

The present work was stimulated by a growing interest among phytoplankton ecologists in accessory pigments and their interactions with physical, chemical and biological factors in the natural environment.

Reynolds (1984) pointed out the need for considering not only common parameters such as light and nutrients, but also others that are often neglected in spite of their recognized importance, possibly because of difficulties of assessment in routine field studies. The accessory pigments come into the latter. They are known to influence the growth of algal populations by their ability in light harvesting and transfer, an especially important fact where light is attenuated and its spectral composition is subjected to changes, as in deep water. On the other hand, they may protect the photosynthetic apparatus from harmful oxygen effects and from photooxydation in surface waters (Clayton, 1980).

It is believed that carotenoids, together with chlorophylls, are the most important plant components that absorb light in aquatic environments (Haxo, 1960; Clayton, 1980). A correlation can therefore be expected between their concentration in the water and algal biomass. Moreover, carotenoids may prove to be more accurate predictors of algal biomass than chlorophyll, because they seem to be less affected than the latter by fluctuations of variables such as light and nutrients (Yentsch and Vaccaro, 1958; Shimura and Fujita, 1975; Foy, 1987; Gieskes et al., 1988).

Field studies on algal carotenoids have been carried out mainly in the sea (Jensen and Sakshaug, 1973; Shimura and Fujita, 1975; Jeffrey, 1981; Leham, 1981). Observations in lakes are very few (Hallegraeff, 1977; Eloranta, 1986; Guilizzoni et al., 1989; Guilizzoni et al., 1990). Moreover, in field studies the carotenoids are generally expressed as a pool, though it has been stressed by several authors that studies on specific pigments can provide valuable information on physiological aspects and on the community structure of the phytoplankton (Margalef, 1965; Hallegraeff, 1977; Eloranta, 1986; Foy, 1987).

The data presented in this paper are part of a larger study on Lake Lugano, focusing on the influence of several physical and chemical factors, and a number of carotenoids, on the photosynthetic activity and the dynamics of the phytoplankton. More details and discussion can be found in Lami (1990).

2. Methods

During 1988 water samplings and primary production measurements (^{14}C method as modified by Gächter and Mares, 1979) were made in a station located in front of the town of Figino (Fig. 1 in Barbieri and Polli, this issue), with a monthly sequence in winter and fortnightly from April to November. Daily production was calculated following Vollenweider (1965) or Gächter (1972). According to the photosynthetic depth profiles, four samples were selected for chemical and ibological analyses: a surface sample (Sup), one at the depth of maximum production (P_{max}), a sample representing the light-dependent part of the photosynthesis curve (P_{min}), and an integrated sample over the whole euphotic zone (Int). In these samples we analyzed the major nutrients and particulate organic carbon (POC, with a CHN analyzer). Chlorophylls and total carotenoids were determined spectrophotometrically, the latter being expressed according to Züllig's (1982) formula. After extraction with 90% acetone, a number of single carotenoids was determined with thin layer chromatography. Plankton carotenoids have been separated in several steps in mixtures of hexane/acetone/isopropanol (Züllig, 1982). Underwater PAR irradiance was measured with a Li-Cor quantum sensor, and water temperature with a thermistor. Algal countings, biovolume and average cell size estimates were made in the P_{max} and Int samples. Backward stepwise multiple regression analysis was performed on $\log_{10}$ transformed data. The criterion required for the exclusion of an independent variable in the regression equation was that the "F-ratio" should be lower than 4.00.

3. Results and discussion

The seasonal variations of the algal biovolume and the pigment concentration are sufficient to demonstrate the persistence of the eutrophic state of Lake Lugano. As

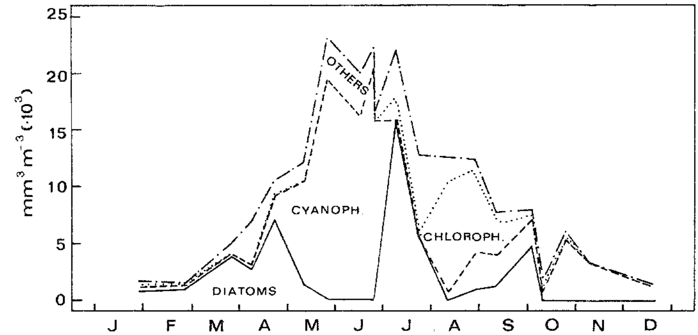

Figure 1. Composition of phytoplankton cumulated biomass in sample P_{max} during 1988.

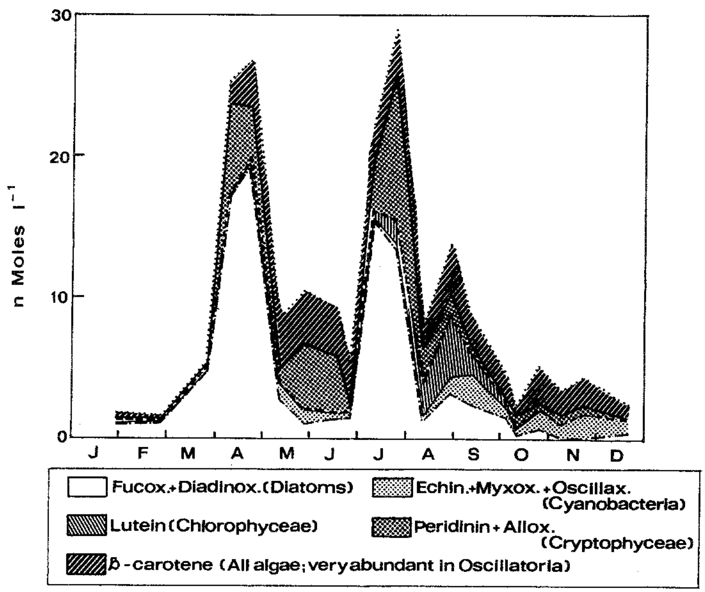

Figure 2. Variation in the concentration (nM l^{-1}) of the most important carotenoids at P_{max}

Figure 1 shows, the biovolume varied from a minimum of 1–2 $cm^3\,m^{-3}$ in winter to a maximum of more than 20 $cm^3\,m^{-3}$ in June at the depth P_{max}. The values found in the integrated samples (1–15 $cm^3\,m^{-3}$) are quite similar to those found at the same station in the last five years (Barbieri et al., 1989). Chlorophyll *a* concentration varied from a minimum of 5 $\mu g\,l^{-1}$ in January to a maximum value of 75 $\mu g\,l^{-1}$ in April, with an annual average of about 25 $\mu g\,l^{-1}$. The range of total carotenoids was 2–29 $nM\,l^{-1}$ (=2–37 $\mu g\,l^{-1}$) over the year (Fig. 2). Notwithstanding the reduction of the nutrient load which is under way for several years (Barbieri and Mosello, this issue), the phytoplankton biovolume did not show any parallel decreases up to the present time. This phenomenon has been found to occur in the near Lake Maggiore (Ruggiu, 1989), and in many other lakes subjected to restoration measures (Sas, 1989). On the other hand, a shift in the community structure seems to be taking place, whereby the diatoms are increasing their overall importance in comparison with the Cyanobacteria (Polli and Simona, this issue).

In 1988 the spring community was in fact dominated by the diatoms, mostly through the strong growth of a small *Stephanodiscus* sp. in March and April, and a lesser growth of *Asterionella formosa* (March) and *Synedra ulna* (April). In May, parallel with the spring diatom decline, and in June, the largest development of the blue-greens took place (Fig. 1). *Oscillatoria redeckei* was the most important species, followed by *O. rubescens*. In June, *O. redeckei* reached values close to 385 10^6 cells l^{-1} in the integrated sample and 625 10^6 cells l^{-1} at the P_{max} depth (1.25 m). The blue-greens collapsed in the epilimnic waters at the beginning of July, and were replaced by a new and stronger diatom growth, with *S. acus* as the prominent species.

The succession proceeded with the Chlorophyta (mainly Chlorococcales) in August. Among these, *Sphaerocystis schroeteri* reached the maximum value of 5.1 10^6 cell l^{-1}, *Tetraëdron minimum* of 19.3 10^6 cell l^{-1}, and *Scenedesmus* sp. of 2.8 10^6 cell l^{-1}. A small development of *Aphanizomenon flos-aquae* occurred in the same period.

The following months were characterized by a further diatom growth in September (*Fragilaria crotonensis*), a general decline of the phytoplankton in early October, and a reappearance of *Oscillatoria* species from mid-October to the end of the year.

Primary production closely followed the seasonal trends of algal densities and biomass. The average daily production was estimated as 1.7 $g\,C\,m^{-2}\,d^{-1}$, with a measured minimum value of 0.20 $g\,C\,m^{-2}\,d^{-1}$ in December and a maximum of 3.36 $g\,C\,m^{-2}\,d^{-1}$ in July. The annual value – estimated as about 550 $g\,C\,m^{-2}$ – corresponds to that of Lake Varese, a much smaller and shallower subalpine hypertrophic lake close to Lake Lugano (Ruggiu et al., 1981). Of all the deep Italian subalpine lakes, Lake Lugano showed the highest primary production values in the past (Ruggiu, 1983). The shape of the production profiles, with very low values (0.19–0.50) of the ratio between the areal and the peak production ($\sum A/A_{max}$) is also typical of eutrophic waters.

Similar courses were observed over the year for chlorophyll and carotenoid concentrations. Their maximum values over the water column corresponded to those of primary production and of POC. The positive correlation ($P < 0.01$) found between POC and both chlorophyll and total biovolume indicates that the origin of in-lake organic matter is essentially autochthonous (unpublished data).

A positive correlation ($P < 0.01$) also exists between total pigments and algal biovolume. However, the peaks of pigments and production, indicating optimal conditions for growth, precede those of biovolume, as recently shown in another lacustrine environment (Guilizzoni et al., 1989, 1990).

In a few studies, specific carotenoids have been analyzed to acquire information on community structure or the seasonal succession of phytoplankton (Gieskes and Kraay, 1984; Eloranta, 1986). In general, in Lake Lugano these pigments proved to be statistically good predictors of algal biomass in the samples Int and P_{max}, especially with diatoms, dinoflagellates and cyanobacteria, and, among the latter, with the genus *Oscillatoria* (Table 1). Of course, this predictability cannot be taken

Table 1. Spearmann Rank correlation coefficients (r) between biovolume of some algal taxa ($mm^3 \times m^{-3}$) and their specific carotenoids ($nM \times l^{-1}$); (* = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$)

	INT	P_{max}
Diatomeae vs Fucoxanthin	0.91 ***	0.085***
Cyanobacteria vs Echinenone	0.78 ***	0.76 ***
Cyanobacteria vs Myxoxanthophyll	0.69 **	0.27 n.s.
Oscillatoria rubescens vs Oscillaxanthin	0.70 **	0.37 n.s.
Cryptophyceae vs Alloxanthin	−0.04 n.s.	−0.13 n.s.
Peridineae vs Peridinin	0.90 ***	0.87 ***
Chlorophyceae vs Lutein	0.53 *	0.43 *

too strictly, because of the known variability in the cellular pigment content according to various factors (e.g., species composition, physiological state, light); in the case of carotenoids these factors have generally been studied in laboratory conditions (Burnett, 1976; Whittingham, 1976). A comparison of Figs. 1 and 2 shows that the biovolume peak in part of May and June is not matched by a peak in the carotenoid concentration. In that period the phytoplankton was strongly dominated by the blue-green *O. redekei*. From the trends shown in the figures it would seem that the specific carotenoid content is lower in the Cyanobacteria than in other algal groups, and, among the species identified in Lake Lugano, is especially low in *O. redekei*. Because a strong correlation ($P < 0.001$) was found between Cyanobacteria biovolume and β-carotene, it can be assumed that this pigment belonged to *O. redekei* in May–June period. Consequently, it can be taken as an index of that algal species biovolume together with the specific carotenoids present.

No substantial differences in pigment concentration over the sampled water column were found. One reason for this is that the euphotic zone is only a few meters thick and easily mixed, so that the algal cells will be submitted to similar average light conditions and no relation between light and pigments will be apparent at any given date. However, significant negative correlation was found between the amount of incident radiation in the two days preceding the experiments and the cellular content of both chlorophyll *a* ($P < 0.01$) and total carotenoids ($P < 0.05$) at the depth P_{max}. Furthermore, a positive correlation was found ($P < 0.05$) between previous incident radiation and I_k. Field data of this kind are not so common in the literature, especially for carotenoids. An example of light adaptation in the field is given by Harris (1978), who found a relationship between I_{opt} values and the sunshine hours in the two days before measured production.

Cluster analysis was used to assess how the considered variables are associated at the different depth levels (Figs. 3 and 4). Nutrients are excluded from both figures, since a preliminary treatment of the data showed that their role on the production variations was insignificant, as could be expected from their great quantity. In Fig. 3 all the remaining variables are presented, while Fig. 4 shows only some selected variables; among these, primary production is introduced as photosynthesis rate per chlorophyll unit (assimilation number AN) and the pigments as cell content, with the carotenoids subdivided into β-carotene and xantophylls.

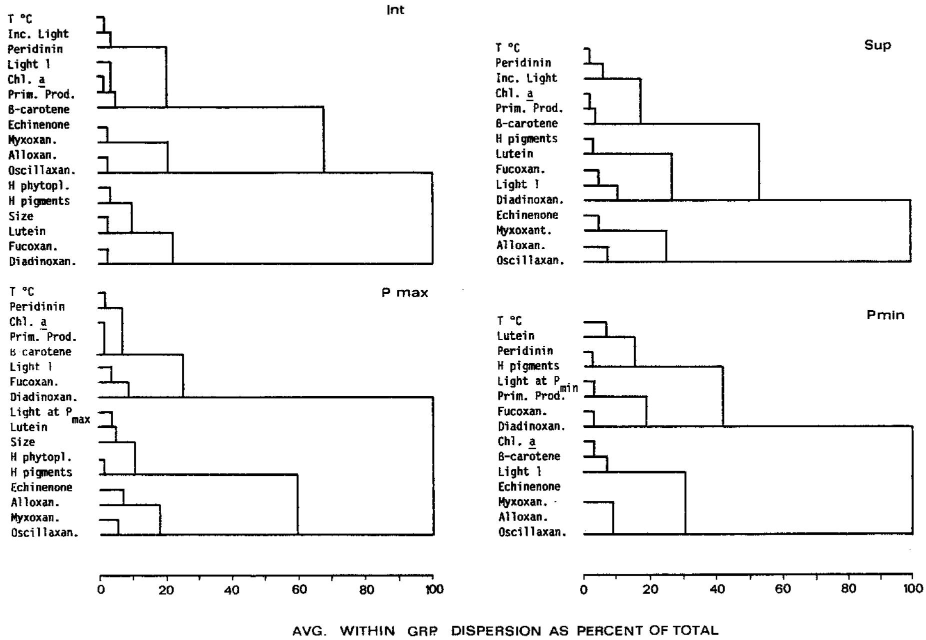

Figure 3. Cluster analysis dendrograms for 4 water samples (Sup, P_{min}, Int, P_{max}) of some physical (temperature, incident light) and bio-chemical variables (pigments as nM l^{-1}; H phyt. = diversity index of phytoplankton; H pigm. = diversity index of pigments; Prim. prod. = primary productivity; size = cell volume). For the sample Int, the mean solar radiation of the previous 2 days of primary productivity experiments is also considered (Light 1)

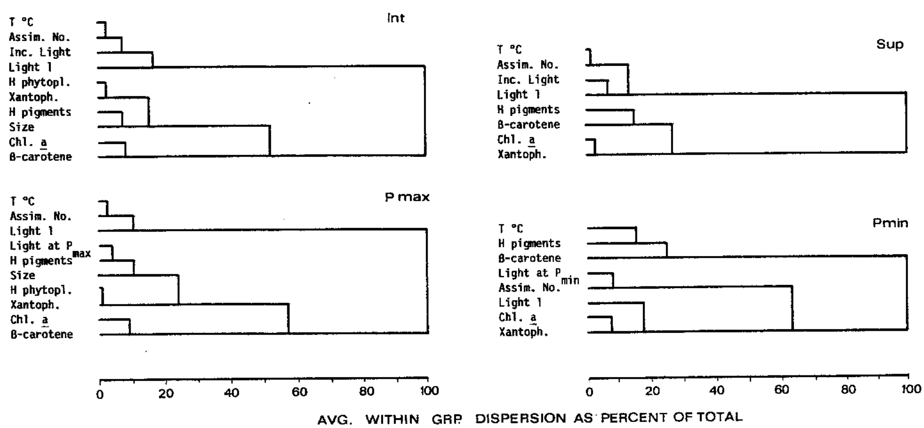

Figure 4. Cluster analysis dendrograms for 4 water samples (Sup, P_{min}, Int, P_{max}) of some physical (temperature, incident light) and bio-chemical variables as content per algal biomass; Assim. No. = assimilation number; size = cell volume; H phyt. and H pigm. = diversity index for phytoplankton assemblages and pigments, respectively). Light 1 as in Fig. 3

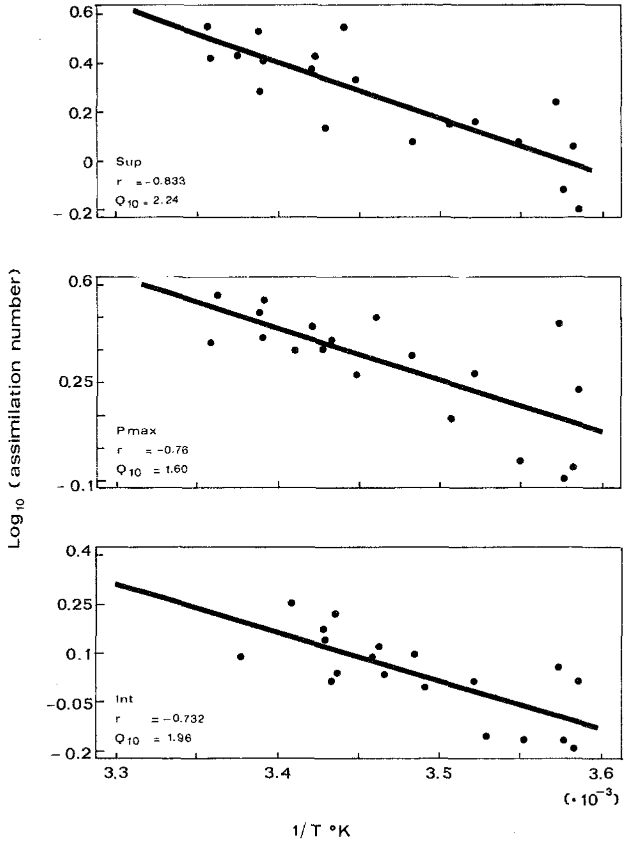

Figure 5. An Arrhenius plot of assimilation number (mg C mg Chl a^{-1} h^{-1}) versus temperature of 3 different water masses (Sup, P_{max}, Int). Correlation coefficients (r) and Q_{10} values are also indicated

From Fig. 3 it can be seen that, with the exception of P_{min}, the photosynthesis rate as mg C m^{-3} h^{-1} is most closely associated with light in the previous days, chlorophyll *a* and some carotenoids (especially β-carotene). Peridinin is often associated with light and temperature, and lutein with temperature and average cell size. AN too is most strongly associated with physical parameters (temperature and light), and secondarily with pigment content (Fig. 4). The specific relationship between AN and temperature is shown in Fig. 5 in the form of Arrhenius plots: Q_{10} values vary between 1.60 at the depth P_{max} and 2.24 at surface.

It was found that in Lake Lugano a set of linear multiple regressions could give a good prediction of primary production. The significance is highest ($r^2 = 0.99$) when light is introduced as the average daily incident radiation in the three days before the experiments, and some carotenoids are also included (Table 2). This proved to be a refinement over a more common approach where chlorophyll *a*, PAR and water temperature are chosen as independent variables: in the case of Lake Lugano, however, these variables were found to explain about 92% of primary production.

Table 2. Regression coefficients and R^2 values of Lake Lugano temperature (°C), PAR ($\mu E\, m^{-2}\, s^{-1}$), total radiation (light two days before experiments; $KJ\, m^{-2}$), chlorophyll *a* and some specific carotenoids ($nM \times l^{-1}$) vs primary production ($nM\, C \times l^{-1} \times hr^{-1}$), backward stepwise regression analysis in the four samples (* = P < 0.05; ** = P < 0.01; *** = P < 0.001)

	INT	SUP	P_{max}	P_{min}
Temperature	0.85***	1.53***	1.03***	n.s.
PAR[1]	n.s.	−0.38**	n.s.	n.s.
Light two days before exp.	0.25***	n.s.	0.23**	−3.15***
Chlorophyll *a*	0.65***	1.35***	1.15***	9.26***
β-carotene	n.s.	−0.36***	−0.45***	−7.90***
Fucoxanthin	n.s.	0.04*	n.s.	−1.07***
Echinenone	−0.085***	n.s.	n.s.	n.s.
Lutein	−0.047**	n.s.	n.s.	n.s.
Oscillaxanthin	−0.048*	n.s.	n.s.	−0.57*
Myxoxanthophyll	n.s.	0.05**	0.04**	n.s.
R^2	0.99***	0.99***	0.99***	0.72**

[1] incident light for INT e SUP

A significant negative linear regression was found between AN and both chlorophyll *a* (Fig. 6) and carotenoid content per unit biomass. A similar relationship was also ascertained in subalpine lakes of different trophic states such as lakes Orta, Mergozzo and Varese (Ruggiu and Morabito, unpublished data). On the other hand, an inverse relationship exists between pigment content and "previous" light, i.e., with the mean total radiation in the two days before the primary productivity experiments. This is in accordance with what is known from classical laboratory experiments, where a shade adaptation results in enhanced pigment synthesis, but also in a lowered ability to use higher irradiances once the adapted algae are exposed to them (see Harris, 1978 for a discussion). Our findings are evidence that varying rates of pigment

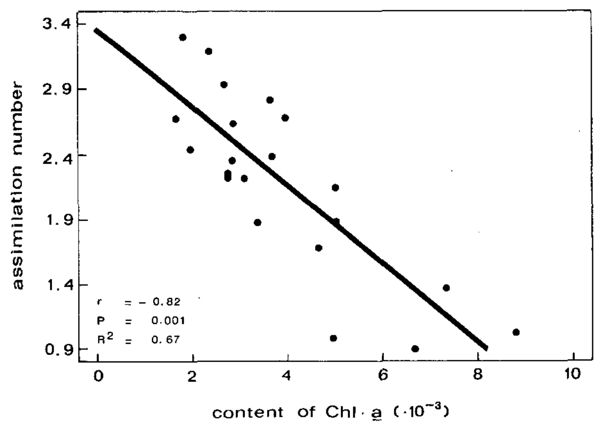

Figure 6. Linear regression between assimilation number (mg C mg Chl a^{-1} h^{-1}) and the content of chlorophyll *a* per unit of biomass ($\mu g\, mm^{-3}$) in the water sample P_{max}

synthesis occur also in the field as a consequence of phytoplankton light conditioning, with ensuing modifications in photosynthetic efficiency. As a result, it appears that the light history is very important in this respect, even though it is often neglected in field studies. Indeed, much more attention should be paid to light or other meteorological conditions preceding the actual measurements than is currently the case in primary productivity studies.

ACKNOWLEDGEMENTS

We are grateful to Mrs. P. Panzani for providing some of the data on algal identification and counts.

REFERENCES

Barbieri, A., B. Polli, G. Righetti e M. Simona, 1989. L'evoluzione trofica del Lago di Lugano in relazione alle misure di risanamento. In: ASTEA (ed.), Giornata informativa del 6/5/1988. Melide, CH, 49 pp.

Burnett, J. H., 1976. Functions of carotenoids other than in photosynthesis. In: T. W. Goodwin (ed.), Chemistry and Biochemistry of Plant Pigments. Academic Press (N.Y.), pp. 655–679.

Clayton, R. K., 1980. Photosynthesis: physical mechanism and chemical patterns. Cambridge Univ. Press (Publ.), 281 pp.

Eloranta, P., 1986. Paper chromatography as a method of phytoplankton community analysis. Ann. Bot. Fennici 23:153–159.

Foy, R. H., 1987. A comparison of chlorophyll *a* and carotenoid concentrations as indicators of algal volume. Freshwat. Biol. 17:237–250.

Gächter, R., 1972. Die Bestimmung der Tagesraten der planktischen Primärproduktion. Modelle und in-situ Messungen. Schweiz. Z. Hydrol. 34:211–244.

Gächter, R. and A. Mares, 1979. Comments to the acidification and bubbling method for determining phytoplankton production. Oikos 33:69–73.

Gieskes, W. W. C. and G. W. Kraay, 1984. Phytoplankton, its pigments, and primary production at a Central North Sea station in May, July and September 1981. Neth. J. Sea Res. 18:51–70.

Gieskes, W. W. C., G. W. Kraay, A. Nontij, D. Setiapernana and Sutomo, 1988. Monsoonal alteration of a mixed and layered structure in the phytoplankton of the euphotic zone of the Banda Sea (Indonesia): a mathematical analysis of algal pigments fingerprints. Neth. J. Sea Res. 22:123–137.

Guilizzoni, P., A. Lami e M. Manca, 1989. Sull'uso di alcuni carotenoidi in ricerche sul plancton e in paleolimnologia. Atti del terzo Congresso Nazionale della Società Italiana di Ecologia, Siena, 21–24 Ottobre 1987:303–308.

Guilizzoni, P., A. Lami, G. Giussani e T. Ruffoni, 1990. Problemi e prospettive dell'utilizzo dei carotenoidi per lo studio dell'evoluzione stagionale del fitoplancton. In: Atti VIII congresso A.I.O.L., Verbania-Pallanza, 31 Maggio-3 Giugno 1988:457–470.

Hallegraeff, G. M., 1977. A comparison of different methods used for the quantitative evaluation of biomass of freshwater phytoplankton. Hydrobiologia 55:145–165.

Harris, G. P., 1978. Photosynthesis, productivity and growth: the physiological ecology of phytoplankton. Arch. Hydrobiol. Beih. Ergebn. Limnol. 10:1–171.

Haxo, F., 1960. The wavelength dependence of photosynthesis and the role of accessory pigments. In: M. B. Allen (ed.), Comparative biochemistry of photoreactive system. Academic Press (N.Y.), pp. 339–360.

Jeffrey, S. W., 1981. An improved thin-layer chromatography technique for marine phytoplankton pigments. Limnol. Oceanogr. 26:191–197.

Jensen, A. and E. Sakshaug, 1973. Studies on the phytoplankton ecology of the Trondheimsfjord. II. Chloroplast pigments in relation to abundance and physiological state of the phytoplankton. J. Exp. Mar. Biol. Ecol. 2:137–155.

Lami, A., 1990. *Alcuni aspetti dell'ecologia del fitoplancton di un lago eutrofo (Lago di Lugano)*. Tesi di dottorato in Ecologia. Università di Parma, 242 pp.
Leham, P. W., 1981. Composition of chlorophyll *a* and carotenoid pigments as predictors of phytoplankton biomass. Mar. Biol. 65:237–244.
Margalef, R., 1965. Ecological correlations and the relationship between primary productivity and community structure. In: R. C. Goldman (ed.), Primary productivity in aquatic environments. Mem. Ist. ital. Idrobiol., Suppl. 18:355–364.
Reynolds, C. S., 1984. Phytoplankton periodicity: the interaction of form, function and environmental variability. Freshwat. Biol. 14:111–142.
Ruggiu, D., 1983. Caratteristiche e comportamento del fitoplancton nei laghi profondi sudalpini. In: C.N.R. AQ/2/20 P.F. Promozione della qualità dell'ambiente. Mescolamento, caratteristiche chimiche, fitoplancton e situazione trofica nei laghi profondi sudalpini, Roma: 71–104.
Ruggiu, D., 1989. Indagini sul fitoplancton. In: Commissione Internazionale per la protezione delle acque italo-svizzere, Rapporto quinquennale 1983–1987, Relazione conclusiva sulle indagini limnologiche relative al Lago Maggiore: 65–77.
Ruggiu, D., C. Saraceni e 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.
Sas, H., 1989. Lake restoration by reduction of nutrient loading: expectations, experiences, extrapolations/coordinations. St. Augustin Academia Verl. Richarz, 497 pp.
Shimura, S. and Y. Fujita, 1975. Changes in the activity of fucoxanthin excited photosynthesis in the marine diatom *Phaeodactylum tricornutum* grown under different culture conditions. Mar. Biol. 33:185–194.
Vollenweider, R. A., 1965. Calculation models of photosynthesis-depth curves and some implications regarding day rate estimates in primary production measurements. Mem. Ist. ital. Idrobiol. 18:425–457.
Whittingham, C. P., 1976. Function in Photosynthesis. In: T. W. Goodwin (ed.), Chemistry and Biochemistry of Plant Pigments. Academic Press (N.Y.), pp. 624–654.
Yentsch, C. and R. Vaccaro, 1958. Phytoplankton nitrogen in the ocean. Limnol. Oceanogr. 3: 443–448.
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.

Received 14 February 1992;
Revised manuscript accepted 24 June 1992.