Hydrobiologia **326/327**: 407–413, 1996.
S. C. Lindstrom & D. J. Chapman (eds), Fifteenth International Seaweed Symposium.

Xanthophyll-cycle and photosynthetic adaptation to environment in macro- and microalgae

Nour-Eddine Rmiki[1], Christophe Brunet[1,2], Jacqueline Cabioch[3], & Yves Lemoine[1,4]
[1]*Laboratoire de Cytophysiologie Végétale et Phycologie, Bât. SN2, UST Lille, F- 59655 Villeneuve d'Ascq Cedex, France*
[2]*Station Marine, U.R.A. 1363, B.P. 80, F-62930 Wimereux, France*
[3]*Station Biologique, UPR 4601, F-29680 Roscoff, France*
[4]*Ecole Normale Supérieure, U.R.A. 1810, F-75230 Paris Cedex 05, France*

Key words: diatoxanthin, *Gracilaria*, photoinhibition, phytoplankton, seaweed, xanthophyll-cycle, zeaxanthin

Abstract

Microalgae and macrophytes adapt their pigment content to the environment because excessive light could limit their photosynthetic rate by inducing photoinhibition. Carotenoids participate in the photoadaptative response especially through the operation of xanthophyll cycles (violaxanthin-zeaxanthin or diadinoxanthin-diatoxanthin). An increasing gradient of diatoxanthin in phytoplankton chromophytes is found from the inshore to the offshore waters, less turbid in relation to the different light penetration in seawater. In addition, a nyctemeral cycle is noted, with a suppression of diatoxanthin at night and its accumulation with the increase of the light. Similarly the vertical distribution, on the French Brittany coasts, of several *Gracilaria* and *Gracilariopsis* species corresponds to increasing zeaxanthin amounts in the seaweeds living at the upper zones, which are more resistant to photoinhibition as shown by fluorescence and oxygen evolution analysis. An operating xanthophyll cycle should be regarded as a regulatory mechanism involved in stress response for the dissipation of excessive excitation energy through de-epoxidated xanthophylls such as zeaxanthin or diatoxanthin.

Abbreviations: Chl – Chlorophyll; Dd – Diadinoxanthin; Dt – Diatoxanthin; F_M – Fluorescence maximum level when PSII centers are closed; $F_{M'}$ – Fluorescence maximum recorded along illumination; F_S – Transitory fluorescence level; F_V – Variable fluorescence; NP_q – Non-photochemical quenching; Q_P – Photochemical quenching.

Introduction

Light, as the energy source, is the essential prerequisite for photosynthesis. However, excess solar energy can lead to photooxidative destruction of the photosynthetic apparatus through photoinhibition (for review see Demmig-Adams & Adams, 1992, and Long et al., 1994). Algae, like higher plants, have developed regulatory mechanisms to avoid photoinhibition which adapt, as far as possible, the rate of energy transfer from the pigment antennae to the rate of transfer of excitation energy to the transducers. If phytoplanktonic algae are able to move through the photic zone and thus to reduce the amount of energy reaching their pigments, macrophytes which are fixed to the substratum need to develop internal mechanisms to increase the dissipation of excitation energy through non- photosynthetic metabolism. Especially under light stress, a process of non-photochemical thermal dissipation of the excess absorbed excitation energy is induced (see Horton et al., 1991).

For several years light-modulated fluorescence has been shown to be a very useful tool to monitor the variations of the photochemical efficiency of Photosystem II (Schreiber et al., 1986). The respective importance of photochemical (Q_P) and of non-photochemical (NP_Q)

energy dissipations can be easily estimated by the analysis of modulated chlorophyll fluorescence upon illumination. When an excess of light energy reaches the photosynthetic apparatus, an increased proportion of absorbed quanta is dissipated as thermal energy (Demmig & Winter, 1988). The main components of this response are associated with the development of the trans-thylakoid pH gradient (Walters & Horton, 1993) and with Photosystem II center's inactivation (Weis & Berry, 1987). Changes in the effective absorption cross-section of Photosystem II may arise from modifications in the pigment composition of the antennae. The so-called 'xanthophyll-cycle' has been observed in higher plants and algae. It consists of the conversion of epoxidated xanthophylls to de-epoxidated derivatives which are normally absent under low fluence densities. The two xanthophyll cycles known up to now concern the β-carotene derivatives violaxanthin, antheraxanthin and zeaxanthin in green, brown and red algae and the allenic carotenoids diadinoxanthin and diatoxanthin in chromophytes (Hager & Stransky, 1970). A strong correlation has been shown between the trans-thylakoid Δ pH, the epoxidation state of the xanthophylls and the non-photochemical quenching of fluorescence (Bilger & Bjorkman, 1990; Henley et al., 1991; Demmig-Adams & Adams, op. cit.; Arsalane et al., 1994).

The work described here concerns the analysis of the importance of the xanthophyll cycle intermediates in the adaptation of various algae to the prevailing light conditions. On one hand, three closely related Rhodophyta with different distributions on the shore have been compared. The highest light tolerance appears to be associated with high proportions of zeaxanthin. On the other hand, correlations have been investigated between the pigment composition of phytoplanktonic algae and the light conditions in the sampling zones. The analysis of samples of the subsurface phytoplankton collected along inshore-offshore transects at various seasons reveal that in high fluence densities the de-epoxidated xanthophyll diatoxanthin is abundant. This study gives additional data showing the protective role of the de- epoxidated xanthophyll cycle intermediates in macrophytes as well as in microalgae.

Materials and methods

The sampled algae were collected in Roscoff (Brittany, France). *Gracilariopsis longissima* (S. G. Gmelin) comb. nov. (Steentoft et al., 1995) from Roscoff grows in the upper inter-tidal level. It can also be collected at a lower level where it is mixed and easily mistaken for *Gracilaria gracilis* (Stackhouse) comb. nov. (Steentoft et al., op. cit.). *Gracilaria multipartita* (Clemente; Harvey) can be found in the sub-tidal level.

After collection, the algae were kept for a few days in the laboratory at 10 °C in 2% P.E.S. enriched seawater (Provasoli, 1968) under illumination 3–60 $\mu Em^{-2}s^{-1}$, depending on their natural environment.

Samples of phytoplankton were collected during 10 oceanographic cruises along inshore-offshore transects on the French coasts of the south-eastern English Channel between Dieppe and Boulogne. At each sampling, the station environmental parameters were measured, i.e. irradiance using a quantometer (Li-Cor, Inc;, Model Li 185B) and turbidity using a turbidimeter (Hach, Model 43 900). Water samples for pigment analysis were taken every 2 nautical miles.

Pigment analysis

The macrophyte pigments were extracted by grinding the thallus in a mixture of methanol: petroleum ether:methylene chloride (80:10:5; V/V). HPLC separation of pigments was realized on a Spherisorb ODS-1 column (Alltech). A slight modification of the gradient system of Gilmore & Yamamoto (1991) was realized with solvent mixture A: methanol-ethyl acetate (68:32; V/V) and B: acetonitrile-methanol-water (72:8:3; V/V). A 0–40% gradient A in A+B allowed the various pigments to elute. Pigments were detected close to their absorption maximum with a Hewlett-Packard 1040 A diode array detector. Previous calibration of the system with standards using the coefficients published by Britton (1985) allowed the quantification of the pigments.

One liter samples of phytoplankton were filtered immediately onto Whatman GF/C filters, which were then stored in darkness and deep-frozen. The pigments were obtained through extraction from the filters in 4 ml of 80% Acetone for 2 h at 4 °C.

HPLC separation of chlorophyll *c* containing plankton extracts was performed using a Beckman ultrasphere RP18 column as previously described (Brunet et al., 1993).

Photosynthetic properties

In vivo light-modulated chlorophyll fluorescence was monitored by a PAM 101 Pulse Amplitude Modula-

tion Fluorimeter (Walz Gmbh, Effeltrich, Germany). Photochemical quenching ($Q_p = (F_{M'} - F_S)/F_{V'}$) and non-photochemical quenching of fluorescence ($NP_Q = (F_M - F_{M'})/F_{M'}$) were calculated as described by Ruban et al. (1993). Oxygen evolution rates of the thalli were assessed simultaneously with a leaf disk Clark type oxygen electrode unit (Hansatech Ltd, G.B.), in a 3% CO_2 enriched atmosphere to prevent any CO_2 limitation of photosynthesis.

Results

Pigment composition of the three tested Rhodophyta

Gracilaria gracilis and *Gracilariopsis longissima* could be observed as mixed populations in the inter-tidal zone on the shores of Roscoff (France). Even though non fertile thalli were difficult to distinguish, these two genotypes had markedly different ecological requirements; the upper zones were occupied by *Gracilariopsis longissima* alone. The pigment analysis of non-photoinhibited *Gracilaria gracilis* thalli revealed that their xanthophyll content was composed of both antheraxanthin and zeaxanthin but lacked the diepoxide violaxanthin. Zeaxanthin amounted to approximately 68% of total xanthophylls and to about 8 molecules on a 100 chl. *a* molecule basis (Table 1).

In contrast, *Gracilariopsis* thalli were absolutely devoid of the monoepoxide antheraxanthin and of violaxanthin. The only xanthophyll was zeaxanthin and its amount corresponded to 16 molecules on a 100 chl. *a* molecule basis (Table 1).

The flat thallus of *Gracilaria multipartita* inhabited the lower inter-tidal zone. The xanthophyll pool was composed of both antheraxanthin and zeaxanthin, the monoepoxide pigment being abundant. Zeaxanthin corresponded to only 55% of total xanthophylls and about 8 molecules on a 100 chl. *a* molecule basis (Table 1).

Photosynthetic properties of the three Rhodophyta

Light saturation curves of oxygen evolution obtained from these closely related seaweeds were different. When light saturation was observed at 200 and 250 $\mu Em^{-2}s^{-1}$ irradiances for *Gracilaria multipartita* and *Gracilaria gracilis* respectively, saturation needed irradiances of 350 $\mu EM^{-2}s^{-1}$ in *Gracilariopsis longissima* thallus regardless of the location on the shore (data not shown).

Photoinhibition

The oxygen exchange activities of thalli from the seaweeds were studied in various light conditions. After a light acclimation period of 15 min under irradiances close to their respective saturation irradiance, the thalli were exposed to a photoinhibiting light of 1590 $\mu Em^{-2}s^{-1}$. If we consider photoinhibition to be reached when oxygen evolution activity is nullified, we may observe that such damage was realized after a 10 min light stress in *Gracilaria gracilis*. Photoinhibition was observed after 15 min illumination in *Gracilariopsis longissima*, collected at the same tidal level, and 22 min in thalli living in the upper zone.

Such a photoinhibition was not accompanied by pigment changes in *Gracilaropsis*, where zeaxanthin was the sole xanthophyll. On the contrary, as shown by data from Table 1, a xanthophyll cycle was operative along the light stress in *Gracilaria gracilis* as well as in *Gracilaria multipartita*. The proportion of antheraxanthin de-epoxided to zeaxanthin corresponded to 69% of its total pool in *G. multipartita* and to 62% in *G. gracilis*.

The light modulated fluorescence properties of these seaweeds were analyzed during the course of a non-saturating illumination (53 $\mu Em^{-2}s^{-1}$) followed by a photoinhibiting treatment, as previously described, and by a 15 min restoration under dim light (53 $\mu m^{-2}s^{-1}$). Table 2 gives the Q_P and NP_Q values for these various seaweeds before photoinhibition and after restoration. It is noticed that the non-photochemical energy dissipation, as indicated by the parameter NP_Q before photoinhibition, was very low in *G. multipartita* (0.25) and *G. gracilis* (0.35) but was higher in *Gracilariopsis longissima*, especially for upper level thalli (0.45). After a 15 min restoration the photoinhibited thalli exhibited an increase of non-photochemical dissipation of excess energy. The NP_Q values exhibited a gradient with the increasing light tolerance of the seaweeds from 3.05 for the shade species *G. multipartita* to 4.38 for *Gracilariopsis* from the upper level (Table 2).

Inshore-offshore variations of pigment composition in phytoplankton

Along inshore-offshore transects (2 m depth), the diatoxanthin content of phytoplanktonic algae showed important variations.. Figure 1 presents the contents of xanthophyll cycle intermediates in algae and turbidity of seawater from 2 to 18 nautical miles off the shore

Table 1. Pigment composition (in nmoles g^{-1} fresh weight) of the 3 Rhodophyta before photoinhibition and at the end of a photoinhibiting light stress (see text).

	Gracilariopsis longissima		*Gracilaria gracilis*		*Gracilaria multipartita*	
	non photoinhibited	photoinhibited	non photoinhibited	photoinhibited	non photoinhibited	photoinhibited
Antheraxanthin	0	0	17.2	6.5	22.8	7.2
Zeaxanthin	67.9	66.9	36.1	46.5	28.3	44.2
Chlorophyll *a*	434.6	426.7	472.4	448.0	338.0	332.0
β-Carotene	14.6	14.5	25.4	23.7	17.6	18.6

Table 2. Levels of the photochemical quenching (Q_P) and non-photochemical quenching (NP*q*) of chlorophyll fluorescence in the various studied Rhodophyta. The measurements have been made before photoinhibition (after 5 min of dim light 53 $\mu Em^{-2}\ s^{-1}$) and in photoinhibited thalli (see Results), after a 15 min restoration in the preceding dim light.

	Photoinhibition			
	Before		After	
	Q_P	NP*q*	Q_P	NP*q*
Gracilariopsis longissima upper level	0.45	0.6	0.23	4.38
Gracilariopsis longissima lower level	0.37	0.43	0.12	3.65
Gracilaria gracilis	0.35	0.41	0.1	3.52
Gracilaria multipartita	0.25	0.28	0.1	3.05

Table 3. Mean pigment ratios in the water column in inshore and offshore stations and irradiances ($\mu Em^{-2}\ s^{-1}$) at 1 m depth measured during the sampling time on 10th May 1990.

	inshore	offshore
Irradiance	300	550
(Dt + Dd)/chla	0.069	0.080
Dd/chl *a*	0.067	0.005
Dt/chl *a*	0.002	0.074
Dt/(Dt + Dd)	0.038	0.930
	n = 12	n = 10

on May 4th 1992. There was a strong reverse correlation between the turbidity of water and the extent of de-epoxidation of diadinoxanthin (R = − 0.86, significance 1%). Equivalent inshore-offshore pigment modifications corresponding to increasing irradiance at 1 m depth were observed on May 10th 1990 (Table 3).

Nyctemeral cycle of the pigment variations in phytoplankton

Daily variations of pigment composition were observed depending on the natural light-dark alternation. Figure 2 shows the pigment variations observed during 24 h on May 6th–7th 1992 along 9 successive transects from the shore to 23 miles offshore. The increase of diatoxanthin from the shore to the open sea, where waters are clearer, could be observed in the afternoon as well as in the morning. At nightfall on May 6th the samples collected 7 miles off the shore contained 8% of the xanthophyll cycle intermediates as diatoxanthin and this proportion slowly decreased to 0 just before midnight, regardless of the sampling zone of the algae. This de-epoxidated xanthophyll was totally lacking during the remaining of the night.

Discussion

The studied Rhodophyta exhibit different pigment compositions. All of them are devoid of violaxanthin so that a possible xanthophyll cycle should be restricted to the final antheraxanthin-zeaxanthin step. Such a xanthophyll cycle is however absent in *Gracilariopsis longissima* from Roscoff as this strain is absolutely devoid of antheraxanthin (Lemoine et al., 1993). This particularity could be considered as a chemotaxonomic marker of this strain and is one more example of the usefulness of individual algal carotenoids as tools for the taxonomists (Weber & Wettern, 1980). Despite such a lack of an operative xanthophyll cycle, *Gracilariopsis* is nevertheless able to colonize upper levels and thus tolerate high fluence densities. The higher light tolerance we have observed in *Gracilariopsis*, as compared to *Glacilaria* species, thus has nothing to do with any xanthophyll de-epoxidation mechanism. Nevertheless, an increase in the amounts of zeaxanthin may be

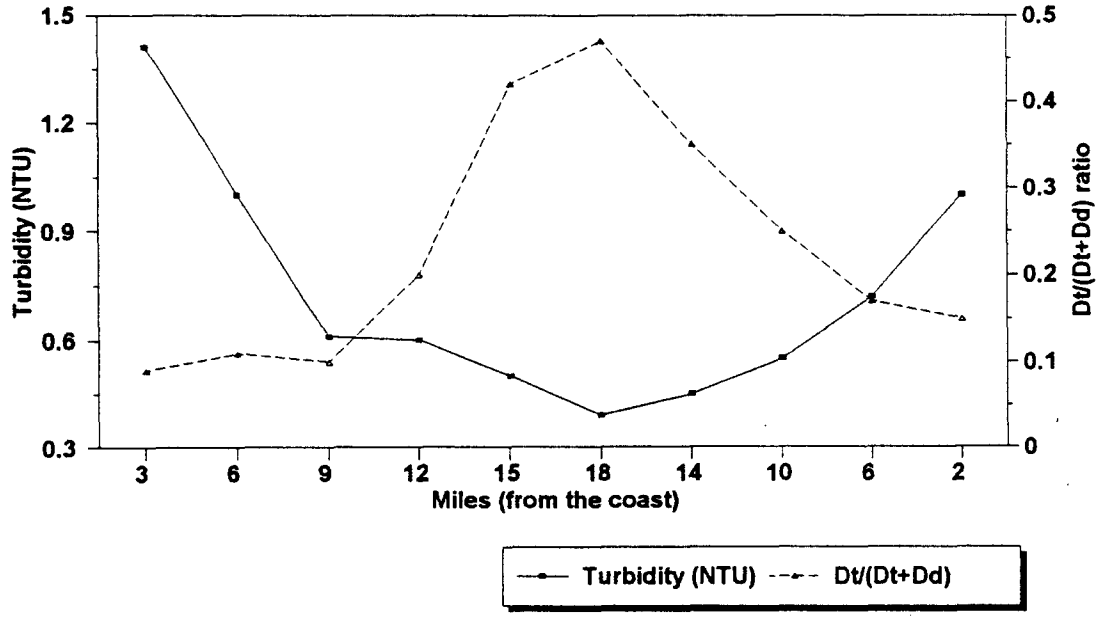

Figure 1. Variations in turbidity (NTU) and Dt/(Dt + Dd) ratio at 2 m depth along inshore-offshore transects carried out on May 4th, 1992. Distance from the coast is expressed in nautical miles.

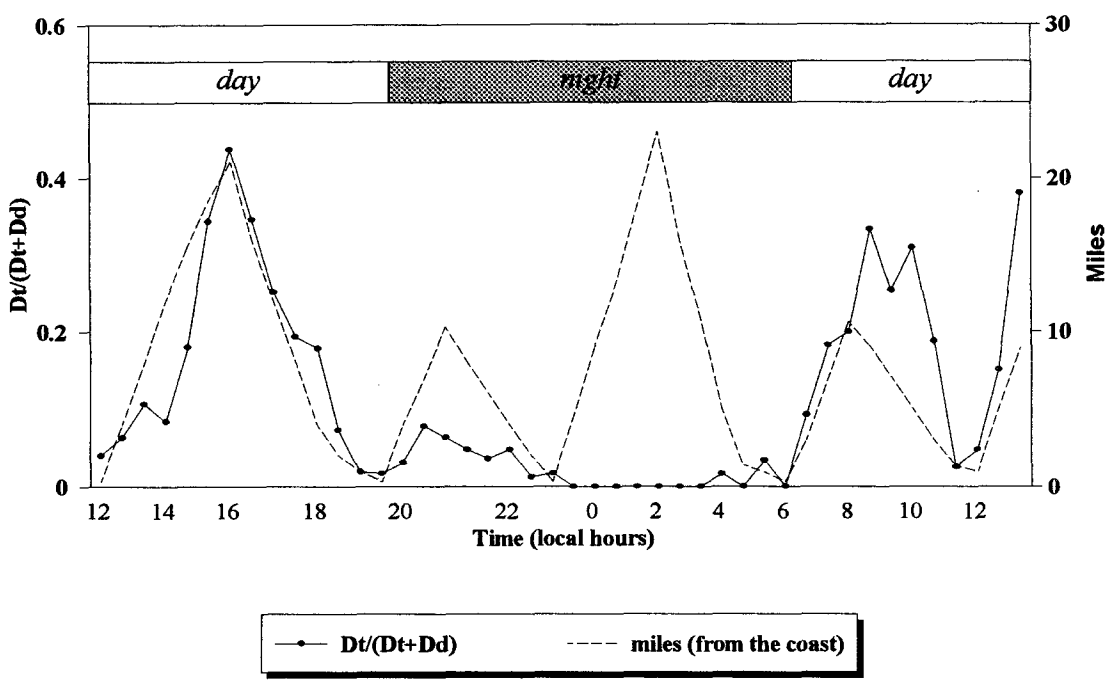

Figure 2. Variations in Dt/(Dt + Dd) ratio along a series of inshore-offshore transects on May 6th–7th, 1992 in the eastern English Channel. Time is in local hours. Distance from the coast is expressed in nautical miles (modified from Brunet et al., 1993).

observed along with an increase in light tolerance in these seaweeds. When the zeaxanthin content corresponds to 55% of the total xanthophylls in the shade-adapted *Gracilaria multipartita* and 68% in *Gracilaria gracilis*, which is absent from the upper intertidal level, it amounts to 100% of the total xanthophylls in the high light tolerant *Gracilariopsis longissima* and is twice as abundant on a chlorophyll molecule basis. A similar increase of the level of the NP_q parameter has been observed (Table 2). This observation is in agreement with the strong correlation between the relative ratio of de-epoxidated xanthophyll (diatoxanthin) and non-photochemical quenching reported by Arsalane et al. (op. cit.) in a diatom culture. The successive distribution of the three Gracilariales from lower to upper intertidal levels probably has its origin in their different abilities to dissipate excessive energy through NP_q. Diurnal photoinhibition and the limitation it can have on production impose a limitation on fitness and survival (Long et al., op. cit.).

In both *G. multipartita* and *G. gracilis* only 62% to 69% of antheraxanthin can be converted into zeaxanthin (Table 1). A similar observation of the existence of 2 pools of epoxidated xanthophylls, only one of which is de-epoxidated through the xanthophyll cycle, has also been reported by Arsalane et al. (op. cit.). The increase of the zeaxanthin content following operation of the xanthophyll cycle is considered to act as a tuner modulating the amount of non-radiative dissipation of energy through aggregation of the light-harvesting complex LHCII (Ruban et al., 1994). Zeaxanthin could also act as a trap for the excess energy by quenching the excited state singlet of chl. *a* (Frank et al., 1994). This process could be favored by structural modifications associated with protonation of lumen-exposed residues on LHCII (Horton et al., 1994). In *Gracilariopsis*, the increase of NP_q occurs without any variation in zeaxanthin content. This increase of NP_q could result from a reorganization of the preexisting zeaxanthin molecules within pigment protein complexes when trans-thylakoid Δ pH increases. Thus, the protective function of carotenoid de-epoxidations does not seem to be associated with the reactions of the xanthophyll cycle *per se* but only with the subsequent formation of zeaxanthin.

Our data concerning phytoplankton show a direct relationship between light and the Dt/(Dt + Dd) ratio indicating an adaptation of phytoplankton to local irradiances. The turbidity of coastal waters is higher because of high river inputs. The inshore-offshore transects lead to clearer waters (Figure 1) and consequently to higher irradiances in the open sea (Table 3). The increase in diatoxanthin under high fluence densities has been reported in cultures of micro algae by Demers et al. (1991). An increase of the time of illumination necessary to abolish net photosynthesis with the increase of the ratio Dt/(Dt + Dd) has clearly been shown in cultures of *Phaeodactylum* by Arsalane et al. (op. cit.). Such a property of diatoxanthin explains the high levels of this xanthophyll that we have observed in the open sea. We have shown the existence of a nyctemeral cycle of diatoxanthin. The persistence of this pigment 3.5 h after nightfall (Figure 2) is equivalent to the low rate of conversion during restoration, already observed for zeaxanthin in higher plants (Foyer et al., 1989).

In conclusion, the operation of the xanthophyll cycle does not appear to be important *per se* since hight light tolerance can be observed even when it is lacking, as shown in *Gracilariopsis longissima* from Roscoff, in which the de-epoxidated pigment (zeaxanthin) that it was expected to produce was already present in high amounts. Therefore, in macrophytes as well as in microalgae, an operating xanthophyll cycle should be considered as a regulatory mechanism supplying de-epoxidated xanthophylls (zeaxanthin or diatoxanthin). These pigments increase excess energy dissipation through non-photochemical quenching of chlorophyll fluorescence. Thus a xanthophyll cycle would not be necessary in algal species rich in carotenoid quenchers which only need to be correctly exposed within the LHCII complexes.

References

Arsalane, W., B. Rousseau & J.-C. Duval, 1994. Influence of the pool size of the xanthophyll cycle on the effects of light stress in a diatom: competition between photoprotection and photoinhibition. Photochem. Photobiol. 60: 237–243.

Bilger, W. & O. Björkman, 1990. Role of the xanthophyll cycle in photoprotection elucidated by measurements in the light-induced absorbance changes, fluorescence and photosynthesis in leaves of *Hedera canariensis*. Photosynth. Res. 25: 173–185.

Britton, G., 1985. General carotenoid methods. In J. H. Law & H. C. Rilling (Eds). Methods in Enzymology. Vol. 111 Steroids and Isoprenoids, Part B, Acad. Press Inc, New-York: 113–149.

Brunet, C., J. M. Brylinsky & Y. Lemoine, 1993. *in situ* variations of xanthophylls Diatoxanthin and Diadinoxanthin: photoadaptation and relationships with a hydrodynamical system in the eastern English Channel. Mar. Ecol. Prog. Ser. 102: 69–77.

Demers, S., S. Roy, R. Gagnon & C. Vignon, 1991. Rapid light-induced changes in cell fluorescence and in xanthophyll- cycle pigments of *Alexandrium excavatum* (Dinophyceae) and *Thalassiosira pseudonana* (Bacillariophyceae) a photo-protection mechanism. Mar. Ecol. Prog. Ser. 76: 185–193.

Demmig-Adams, B. & W. W. III Adams, 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant. Physiol. Plant mol. Biol. 43: 599–626.

Demmig, B. & K. Winter, 1988. Characterisation of three components of non-photochemical fluorescence quenching and their response to photoinhibition. Aust. J. Plant Physiol. 15: 163–177.

Foyer, C., M. Dujardyn & Y. Lemoine, 1989. Responses of photosynthesis and the xanthophyll and ascorbate-glutathione cycles to changes in irradiance, photoinhibition and recovery. Plant Physiol. Biochem. 27: 751–760.

Frank, H. A., A. Cua, V. Chynwat, A. Young, D. Gosztola & M. R. Wasielewski, 1994. Photophysics of the carotenoids associated with the xanthophyll cycle in photosynthesis. Photosynth. Res. 41: 389–395.

Gilmore, A. M. & H. Y. Yamamoto, 1991. Resolution of lutein and zeaxanthin using a non-endcapped lightly carbon-loaded C18 high performance liquid chromatographic column. J. Chromatogr. 543: 137–145.

Hager, A. & H. Stransky, 1970. Das Carotenoïdmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen. V. Einzelne Vertreter der Cryptophyceae, Euglenophyceae, Bacillariophyceae, Chrysophyceae und Phaeophyceae. Arch. Mikrobiol. 73: 77–89.

Henley, W. J., G. Levavasseur, L. G. Franklin, B. D. Osmond & J. Ramus, 1991. Photoacclimation and photoinhibition in *Ulva rotundata* as influnced by nitrogen availability. Planta 184: 235–243.

Horton, P., A. V. Ruban, D. Rees, A. A. Pascal, G. Noctor & A. Young, 1991. Control of the light-harvesting function of chloroplast membranes by aggregation of the LHCII chlorophyll-protein complex. FEBS Lett. 292: 1–4.

Horton, P., A. V. Ruban & R. G. Walters, 1994. Regulation of light harvesting in green plants. Indication by non-photochemical quenching of chlorophyll fluorescence. Plant Physiol. 106: 415–420.

Lemoine, Y., N.-E. Rmiki, G. Zabulon & J. Cabioch, 1993. Carotenoid composition and photoinhibition in several related red algae. Xth International Symposium on Carotenoids, Trondheim (Norway), p. 6–5.

Long, S. P., S. Humphries & P. G. Falkowski, 1994. Photoinhibition of photosynthesis in nature. Ann. Rev. Plant. Physiol. Plant mol. Biol. 45: 633–662.

Provasoli, L., 1968. Media and prospects for cultivation of marine algae. In A. Watanabe & A. Hattori (eds). Cultures and collections of algae. Proc. U.S.-Japan Conf., Hakone. Jpn. Soc. Pl. Physiol., pp. 63–75.

Ruban, A. V., A. J. Young & P. Horton, 1993. Induction of nonphotochemical energy dissipation and absorbance changes in leaves. Evidence for changes in the state of the light- harvesting system of Photosystem II *in vivo*. Plant Physiol. 102: 741–750.

Schreiber, U., U. Schliwa & W. Bilger, 1986. Continuous recording of photochemical and non photochemical fluorescence quenching with a new type of modulation fluorometer. Photosynth. Res. 10: 51–62.

Steentoft, M., L. M. Irvine & W. F. Farnham, 1995. Two terete species of *Gracilaria* and *Gracilariopsis* (Gracilariales, Rhodophyta) in Britain. Phycologia 34: 113–127.

Walters, R. G. & P. Horton, 1993. Theoretical assessments of alternative mechanisms for non-photochemical quenching of PS II fluorescence in barley leaves. Photosynth. Res. 36: 119–139.

Weber, A. & M. Wettern, 1980. Some remarks on the usefulness of algal carotenoids as chemotaxonomic markers. In F. C. Czygan (ed.). Pigments in plants. Gustav Fischer Publ. Stuttgart — New-York, pp. 104–116.

Weis, E. & J. Berry, 1987. Quantum efficiency in relation to energy dependent quenching of chlorophyll fluorescence. Biochim. biophys. Acta 283: 259–267.