THE RESPONSE OF *PERIDINIUM GATUNENSE* TO DIFFERENT LIGHT REGIMES: ITS PIGMENTATION AND POSSIBLE IMPLICATIONS ON THE LIGHT-SHADE ADAPTATION

LIU Xue-jun[1] and Yosef Z. YACOB[2]

(1. *Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan* 430072, *China*.; 2. *The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic & Limnological Research Ltd, Tiberias* 14102, *Israel*)

Abstract: The response of *Peridinium gatunense* (formerly *Peridinium cinctum* fa. *westii*) to different light regimes was investigated based on pigment profiles, which were revealed by HPLC analyses, over the ranges of 16.6-250μE · m^{-2} · S^{-1}. Peridinin and diadinoxanthin are the two most important carotenoids, shared about 90% of the total xanthophylls. Cellular chlorophyll a showed an evident drop along the increased irradiance gradients. Evidence was presented to show the role of diadinoxanthin and β-carotene as light protectants, including: 1) diadinoxanthin / chlorophyll a ratios increased drastically when *Peridinium* was exposed to increased light gradients and β-carotene / chlorophyll a ratios also showed an obvious increasing tendency; 2) β-carotene / chlorophyll a ratios under light condition were higher than those under completely dark condition; and 3) diadinoxanthin / chlorophyll a ratios exhibited inter-group differences among high irradiance, low irradiance, high to low and low to high irradiance treatments. Hereby the possible implication for pigment metabolic routes and their limnological behaviour was deduced.

Key words: Lake Kinneret; Light-shade adaptation; *Peridinium gatunense*; Pigmentation

CLC number: Q0745.11 **Document code**: A **Article ID**: 1000-3207(2000)04-0307-10

Lake Kinneret (32° 42′ to 32° 55′ N, 35° 31′ to 35° 39′ E), the only large freshwater lake and the regulation reservoir in the National Water Carrier Project in Israel, is limnologically characterized by annual occurrence of spring water-bloom of the dinoflagellate *Peridinium gatunense* (formerly *P. cinctum* fa. westii), during which, *Peridinium* biomass usually shared about 94%— 99% of the total phytoplankton volume[1], [2], [3], with patches sometimes up to 4,000 cells · ml^{-1}[2]. Field investigations showed that one conspicuous feature of *Peridinium* cells was their motility, particularly vertical migration tendency to the deep layer in the water column. This active migration, combined with wind-driven water mass movement, allowed them to

收稿日期: 1997-09-08; **修订日期**: 1999-11-22

基金项目: 国家自然科学基金资助(编号: 39770146)

作者简介: 刘学君(1966—), 男, 湖北省仙桃市人, 现在香港城市大学读博士

show a special vertical distribution pattern. Most investigators regarded it as an induced reaction to strong irradiances in the water surface. We could assume that *Peridinium* cells appeared to succeed in maintaining a huge population via this kind of photoadaptation although nutrient availability is very critical in maintaining the size of the population. Unfortunately, so far the existing data could not provide solid evidences on the assumption, there still remained nuknown from physiological point of view that to what extent the irradiances affect their limnological behavior and what the feedback is, namely, how to show their coordination with the light gradients.

The general objective of the present study is to reveal the pigment composition (or dynamics) during laboratory culture over a light range of 16.6—250μE · m^{-2} · s^{-1}, and show the discrepancies between the light-adapted *Peridinium* cells and shade-adapted ones. It is expected to explain the field pigmentation in the Lake Kinneret and provide some implications on the metabolic mechanism among the pigment species.

1 Materials and Methods

1.1 Batch cultures of *P. gatunense* The cultures were established by incubating ca. 50mL of exponential growth phase culture into several Erlenmeyer flasks containing 1,500mL Lindstrom culture medium[4] in order to obtain a reasonable initial cell numbers, and illuminated continuously with cool white fluorescent tubes (Osram L40W/10S, Germany). To yield the different irradiances, we adjusted the distance from light sources to cultures. The irradiances were examined by means of a Biospherical light meter (QSL-100) equipped with a spherical silicone sensor. Each treatment had three replicates. The cultures were adapted at 17℃ and not aerated, but shaken gently once daily so as to prevent sedimentation and artificial breakage of the cells.

Experiment 1 Considering the low initial cell density, the culture experimentation lasted 34 days, during which several milliliters of culture were taken to check the growth at regular intervals, depending on the cell numbers. The growth curves were expressed simultaneously in terms of *in vivo* fluorescence values (arbitrary values at a combination of 31.6 scale and × 1 sensitivity, Turner Designs Fluorometer) and cell numbers. The cell number of each flask was counted using a Wild Heerbrugg inverted microscope (Switzerland), at least three subsamples being used. Finally the growth curves were monitored in terms of cell density and *in vivo* fluorescence values. The range of light gradient was 25—250μE · m^{-2} · s^{-1}.

Experiment 2 The cultures above were harvested at the 28th day to show the differences between the light-adapted and shade-adapted *Peridinium* cells in light-harvesting accessory and photosynthetic pigments. The light employed was over the range of 16.5—250μE · m^{-2} · s^{-1}.

Experiment 3 To elucidate the role of diadinoxanthin, β-carotene and chlorophyll a in the light adaptation, we transferred the cultured *Peridinium* cells from light to dark and *vice versa* with controls (exposure to light and dark environments throughout the whole experimentation, respectively). Also, the dynamics of β-carotene / chlorophyll a ratios and diadinoxanthin / chlorophyll a ratios was observed during Experiment 1, *viz.* in the range of 25—250 $\mu E \cdot m^{-2} \cdot s^{-1}$.

1.2 HPLC analyses of pigment composition Cultures were sampled at 5—10 day interval for HPLC analysis of the chloropigments and some predominant carotenoids. A 50—100mL culture was filtered onto Whatman GF / C glassfiber filters under subdued light and low negative pressure. The filters were folded into two sectors and kept in the freezer at −18℃ until all sampling finished. All filters were sonicated using an electronic homogenizer equipped with a Teflon pestle in cold 5 + 5 mL 90% acetone solution. Let the suspension stand in the dark room (4℃) overnight. Prior to HPLC operation, the extract solutions containing residues were centrifuged and passed through GF / F glassfiber filters installed in a syringe if necessary. To obtain better resolution among the hydrophilic pigments, 0.3ml mol / L ammonium acetate (served as the function of an ion-pairing reagent) was added to 1.0mL clear acetone extract[5], mixed well and let it equilibrate for at least 5 minutes before injection. The HPLC method used was the modification from Literature[5] [6]. HPLC system used was the modification similar to that of Yacobi et al. [18]. The detection was performed at 436nm in Milton Roy variable wavelength detector. The column we employed was reverse-phased Phenomenex Spherisorb 5μ, C_{18}, 250mm length and 4.6mm inner diameter (U.S.A), and its initial working conditions were flow rate at $1.5mL \cdot min^{-1}$, pressure at ca. 1,500psi. Three solvents in the gradient elution were used: A) 30% 1mol / L ammonium acetate (Aldrich Chemical Company, U. S. A) in the water purified with Barnstead ultrapure water system and 70% absolute methanol (HPLC grade, Bio-Lab Ltd. Israel); B) 30% ethylacetate (HPLC grade, Bio-Lab Ltd, Israel) and 70% methanol; and C) 100% methanol for washing the system after each working day. A ternary procedure was designed. Each run took 20 minutes. Chromatographic separations were obtained by the first linear gradient elution from 20% B solvent to 60% B solvent in 5 minutes, followed by an isocratic hold at the combination of 40% A and 60% B, and the second linear gradient elution from 60% B to 100% B, followed by a single solvent B for 5.0 minutes. During the interval between runs, the instrument and the elution solvent were initialized.

The eluted fractions were qualified and quantified according to the method of [7].

2 RESULTS

2.1 Growth curves Judging from the growth curves in terms of both *in vivo*

fluorescence value and living cell numbers (cell density)(Figs. 1a and 1b), *Peridinium* displayed several obvious phases in the cultures. About 20 days after the beginning of cultivation, these cultures, with no exceptions, reached their peak biomass, although the *in vivo* fluorescence values decreased thereafter sharply, which presumably was due

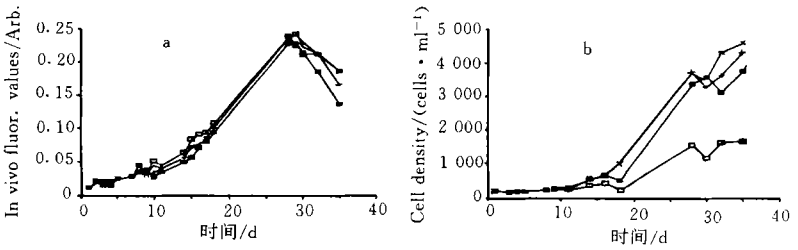


Fig. 1 *P. gatunense* growth curves over the range of 25, 86, 125, 250μE · m^{-2} · s^{-1}(图示为 ×－×, ＋－＋, －□－, －■－). a: in terms of *in vivo* fluorescence values (arbitrary); b: in terms of living cell densities (cells / mL).

to the increase of senescent cells in the whole population. Thus we inferred that these cultures should have been in their late-logarithmic phases in terms of cell density. It was meaningful to note that at 25μE · m^{-2} · s^{-1} (lowest irradiance) the peak population was so low that it only accounted for one fourth of the peaks from other three treatments although it was not obvious in *in vivo* fluorescence value. It seemed that the intrinsic increasing capability of this population (cell number) was obviously restricted due to the very dim light.

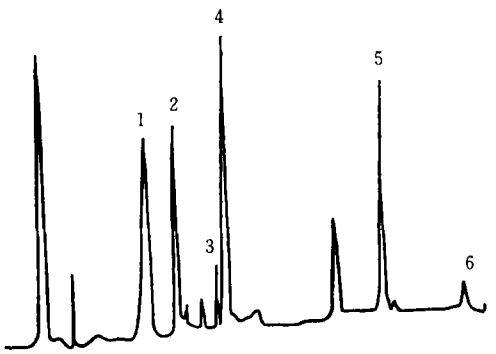


Fig. 2 A typical HPLC absorbance chromatogram detected at a wavelength of 436nm (run time= 20.01min.). Identified pigments 1: chlorophyll *c* [retention time (rt) =6.16min.]; 2: peridinin (rt=7.33min.); 3: dinoxanthin(rt=9.08min.); 4: diadinoxanthin (rt=9.43min.); 5: chlorophyll a (rt=15.80min.); 6; β-carotene (rt = 19.06min.).

2.2 Identified pigments A typical reverse-phase chromatogram is shown in Fig. 2, demonstrating the peaks of the major pigments and relative retention time. Our system recognized all the pigments typically belonging to Peridiniaceae, Peridiniales[8, 9]. This pigment suite consisted of chlorophyll a [(λ_{max}(methanol) = 433, 665nm], peridinin [(λ_{max}(methanol) = 475nm], diadinoxanthin [(λ_{max}(methanol) = 422, 447, 476nm], dinoxanthin [(λ_{max}(methanol) = 418, 442, 470nm], chlorophyll *c* [λ_{max}(methanol) = 445, 632nm] and β-carotene [λ_{max}(methanol) = 451, 480nm] in the order of their polarity.

2.3 Cellular pigments and ratios of different pigment species to chlorophyll a The data pertaining to cellular pigment composition were presented in Fig. 3a and the

pigment ratios to chlorophyll a in Fig. 3b. Chlorophyll a is the center for photosynthetic reaction, and is certainly the major light-harvesting pigment. At subdued light conditions (at 16.6, 35.2 in experiment 2 and at 25μE • m^{-2} • s^{-1} in experiment 1), chlorophyll a was at its maximum. The same tendency, *viz.* chlorophyll a decreased along the light gradient, occurred during the two experimentations. It should be noted that cellular chlorophyll a was higher than 500pg per living cell at 16.6250μE • m^{-2} • s^{-1}, which was about 4.2 times higher than that at 250μE • m^{-2} • s^{-1}(see Fig. 3a); Peridinin and diadinoxanthin, undoubtedly the two

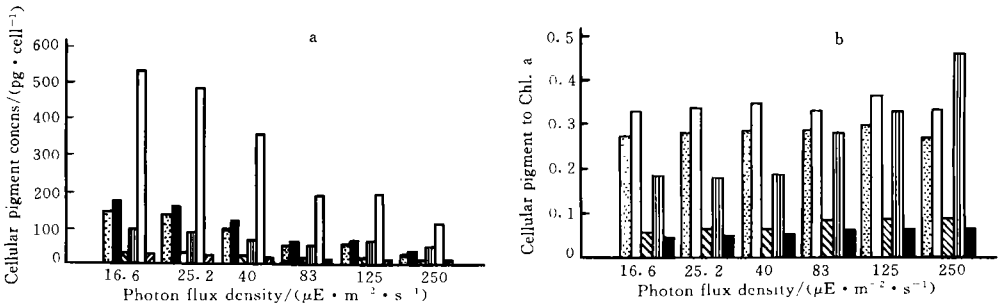


Fig.3 Cellular concentrations of major photosynthetic pigments (a) and their relative proportions against chlorophyll a (b).

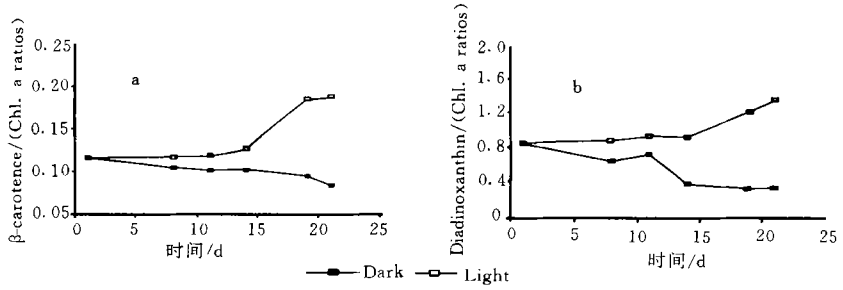
chl.c ■perid ▨ dinox　　chl. c/a □ perid/chla　　dinox/chl.a

diadinox □chl.a ▨ β-carot　　diadinox / chl.a　　■β-carot / chl.a

most important carotenoids, shared about 90% in the total xanthophyll. Along with the increasing irradiances, the cellular chlorophyll a showed an evident drop regularly. Cellular peridinin also decreased with the increase of irradiance, which appeared to be very much related to the metabolic equilibrium between dinoxanthin and peridinin. In addition, the increase in cellular peridinin led to the stability of peridinin / chlorophyll a ratios (Fig. 3b). On the contrary, diadinoxanthin / chlorophyll a ratios increased sharply. The value at 250μE • m^{-2} • s^{-1} was 1.4 times higher than that at 16.6μE • m^{-2} • s^{-1}.

Further evidences on the role of diadinoxanthin and β-carotene as photoadaptation pigments were shown in Figs. 4a, 4b and 4c. β-carotene / chlorophyll a ratios in light treatment was quite higher than that at dark condition, and there was significant difference between them from statistic viewpoint (t-test, $P < 0.01$). Diadinoxanthin / chlorophyll a ratios exhibited nearly the same tendency with a threefold increase under the light condition. In the transition experiments, ratios of diadinoxanthin to chlorophyll a at both high-to-low and low-to-high treatments were statistically higher than those in their controls (under high light condition and low light condition throughout the whole experimentation, respectively, see Fig.4c).

In the range of 25—250 μE • m^{-2} • s^{-1} (Experiment 3), Figs. 5a and 5b



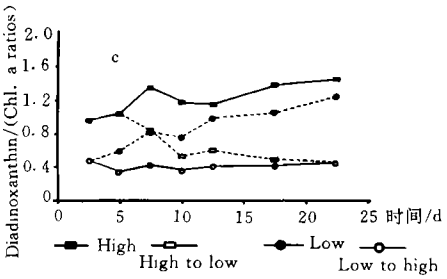


Fig. 4 β-carotene and diadinoxanthin ratios to chlorophyll a during different experimentation. 4a: β-carotene / chlorophyll a ratios under dark and light conditions; 4b: diadinoxanthin / chlorophyll a ratios under dark and light conditions; 4c: diadinoxanthin/chlorophyll a ratios during transfer experimentation.

demonstrated that both β-carotene / chlorophyll a and diadinoxanthin / chlorophyll a ratios showed the similar tendency: values at high irradiances were much greater than at low light treatment. But at 250μE • m^{-2} • s^{-1} and 125μE • m^{-2} • s^{-1} groups, the diadinoxanthin / chlorophyll a ratios leaped irregularly.

3 DISCUSSION

The similarity in the growth curves among different light regimes displayed in the present study suggested that all cultures

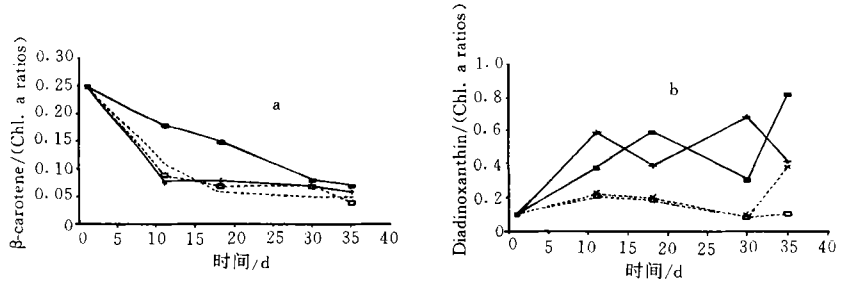


Fig. 5 β-carotene and diadinoxanthin ratios to chlorophyll a over the range of 25—250μE • m^{-2} • s^{-1}. 5a: β-carotene ratios to chlorophyll a; 5b: diadinoxanthin ratios to chlorophyll a.
—■—250μE • m^{-2} • s^{-1} —□— 125μE • m^{-2} • s^{-1} — + — 86μE • m^{-2} • s^{-1} — × — 25μE • m^{-2} • s^{-1}

be at or near light saturation, resulting from light adaptation (to be published elsewhere). The decrease in *in vivo* fluorescence values at 250, 125 and 86μE • m^{-2} • s^{-1} may be caused from cell aging and probably nutrient deficiency in the cultures. Another reason for this, as Brown *et al.*[10] pointed out, might be that the cultures were not aerated and availability of carbon dioxide was likely the limiting factor for biomass.

Bianchi and Findlay[11] draw a conclusion from their pigment measurements in the emergent and submerged macrophytes that emergent plant yielded a high concentration of β-carotene, and this was the reason for the fact that they were exposed to the direct sunlight, in which case it was necessary to preserve such a high concentration of photoprotective pigment. Except excessive irradiance protection and UV and near-UV injury protection[12], β-carotene accumulation also prevented algal cells from the environmental stress conditions such as nutrient availability limitation (Ben-Amotz[13] and the references therein). Our results strongly supported this opinion. At relatively high irradiances, as Fig. 5a showed, the β-carotene / chlorophyll a ratios exceeded the values at $25\mu E \cdot m^{-2} \cdot s^{-1}$ by 4—4.5 times, but the light-induced variations exhibited in all four treatments were quite uniform, namely, from the beginning of inoculation to harvesting, the ratios decreased sharply. We assume that there might be two reasons with respect to this event: 1) owing to the exposure to the low light for a long time, *Peridinium* cells showed the obvious physiological reaction (shade-adaptation) by decreasing the ratios; 2) along with the division and breakdown of the cells, the culture had increasing turbidities, which resulted in the attenuation of the average light levels above the flasks and which, according to the data reported, can rise up to 20%—30% of the initial light density.

As described above, the cellular chlorophyll a of *Peridinium* is surprisingly higher than any results previously published[14]. According to Pollingher and Serruya[3], *Peridinium* cells fall into four growth categories as a response to defined environments: giant, larger, normal and small ones. At *in situ* investigation, the giant and larger cells occurred at the onset of the *Peridinium* bloom, after that a lot of encysts were formed and the normal and small cells dominated the *Peridinium* population. Because of the similarity between the laboratory culture and field situation while a heavy *Peridinium* bloom existed (the so-called uni-algal ecosystem), the results may be applied to the culture to interpret the events of the chlorophyll a concentration per cell. In the present study, the suppression from the relatively low irradiance, to whatever extent, existed. It is plausible to state that before the senescent phase of the culture, chlorophyll a kept increasing more or less. However, in the late-logarithmic or senescent phase of culture, due to the likely nutrient limitation, not so healthful physiological states and the accumulation of the excreted organic materials (self-inhibition), the division rates substantially were reduced and the mortality and breakdown process were very fast and largely intensified[15]. Solid evidences already existed to indicate that chlorophyll a remains intact in the culture without further degradation to any other forms such as chlorophyllide and pheophytin since the induced *in vivo* chlorophyllase activity was very low during the cell breakage (Unpublished research proposal in 1994). Consequently, the quotient of chlorophyll a

content in the culture to the living cell numbers was of course dropped owing to the fact that a lot of debris containing large portions of chlorophyll a contributed by those giant and larger cells existed. The cellular chlorophyll a was unexceptionably related to light levels. The following equation can express the relationship between the two parameters in logarithmic phase: $C = 1044.8-406.0 \times \log I (r=0.96, p = 0.01)$, where C and I were designated as cellular chlorophyll a content and irradiances, respectively.

Continuous raise of peridinin was also found in the field observation until cell aging and possible encystation towards the end of the bloom[16]. This fact leads to the assumption that there might be some biosynthetic pathways allowing other carotenoid(s) convert into peridinin. From the present experiments, whether or not there are derivatives in the route of peridinin biosynthesis from dinoxanthin (the precursor of this signature pigment belonging to the dinofiagellates) due to the incorporation of atmospheric oxygen remained unclear. Probably, *Peridinium* has two cycles among xanthophylls, one is the widely existed epoxidase-deepoxidase reaction in the Division Eukaryota. In this enzymatic reaction, one side of the equilibrium is diadinoxanthin (exoxy form), the other might be other xanthophyll(s) below the detection limit, which occurred only in margin quantity; the second appeared to be dinoxanthin ⇔ peridinin in stead of zeaxanthin ⇔ antheraxanthin ⇔ violaxanthin in the case of *Peridinium*[17]. Fig. 6 shows peridinin / dinixanthin ratios along the irradiance gradient, from which it is suggested that the low light illumination could promote the biosynthesis of peridinin.

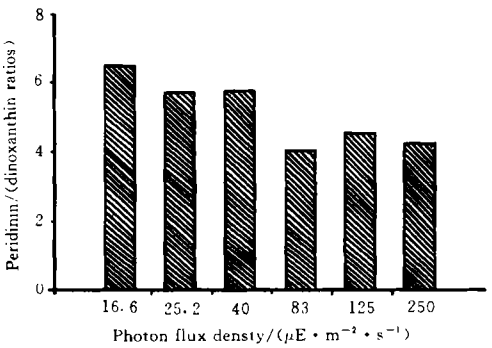


Fig.6 Peridinin/dinoxanthin ratios over the range of 16.6—250μE · m^{-2} · s^{-1} in the logarithmic phase of *Peridinium* cultures.

From the results above, the low irradiances could promote the two carotenoid cycles postulated above to diadinoxanthin and peridinin formation. Previous investigations showed that peridinin and dinoxanthin, relative to β-carotene, were easily degraded to their end products before they reached the water-sediment interface[15],[18]. It should be very interesting to assume that there is a junction between the two metabolic pathways, in other word, one is the bypath affiliated with the other (the dominant pathway). But further evidences are necessary to substantiate this assumption, and a long quest deep into the oracle of carotenoid metabolism could be desired.

Acknowledgements We would like to extend our thanks to Prof. T. Berman

and his staff members at the Kinneret Limnological Laboratory, Israel Oceanographic and Limnological Research Ltd (Israel), who kindly allowed the authors use their facilities and were always ready to help, and Prof. Liu Jiankang for his critical review of the draft manuscript. Thanks are also due to the Society for Transfer of Technology (Jerusalem), the Ministry of Foreign Affairs of Israel for providing the fellowship to Liu Xuejun.

This work was partly granted by the National Natural Scientific Foundation of China (contract No: 39770146, to Liu Xuejun).

REFERENCES

[1] Pollingher U, Berman T. Temporal and spatial patterns of dinoflagellate blooms in lake Kinneret, Israel (1969—1974) [J]. *Verh. Internat. Verein. Limnol.* 1975, **19**: 1370—1382

[2] Beman T, Rodhe W. Distribution and migration of *Peridinium* in Lake Kinneret [J]. *Mitt. Intemat. Verein. Limnol.* 1971, **19:** 266—276

[3] Pollingher U, Serruya C. Phased division of *Peridinium cinctum* f. westii (Dinophyceae) and development of the Lake Kinneret (Israel) bloom [J]. *J. Phycol.* 1975, **12:** 162—170

[4] Lindstrom K. Effect of temperature, light and pH on growth, photosynthesis and respiration of the dinoflagellate *Peridinium cinctum* fa westii in laboratory culture [J]. *J. Phycol.* 1984, **20**: 212—220

[5] Zapata M. et al. Separation of chlorophylls and their degradation products in marine phytoplankton by reversephase high-performance liquid chromatography [J]. *Chromatographia*, 1987, **23**: 26—30

[6] Wright S W et al. improved HPLC method for the analysis of chlorophylls and cartenoids from marine phytoplankton [J]. *Mar. Eco. Prog. Ser.* 1991, **77:** 183～196

[7] Mantoura R F C, Llewellyn C A. The rapid determination of algal chlorophyll and carotenoid pigments and their breakdown products in natural waters by reverse-phase high-performance liquid chromatography [J]. *Analytica Chemica Acta*, 1983, **151**: 297—314

[8] Johansen J E, et al. Carotenoids of the Dinophyceae [J]. *Phytochemistry*, 1974, **13**: 2261—2271

[9] Jeffrey S W. Chloroplast pigment patterns in Dinoflagellates [J]. *J. Phycol.* 1975, **11**: 374—384

[10] Brown M R. et al. The influence of irradiance on the biochemical composition of the *Prymnesiophyte Isochrysis* sp. (clone t-iso) [J]. *J. Phycol.* 1993, **29**: 601—612

[11] Bianchi T S, Findlay S. Plant pigments as tracers of emergent and submergent macrophytes from the Hudson River [J]. *Can. J. Fish. Aquat. Sci.* 1990, **47**: 492—494.

[12] Paerl H W. Cyanobacterial carotenoids: their roles in maintaining optical photosynthetic production among aquatic bloom forming genera [J]. *Oecologia*, 1984, **61:** 143—149

[13] Ben-Amotz A. Effect of irradiances and nutrient deficiency on the chemical composition of *Dunaliella bardawil* Ben-Amotz *et* Avron (Volvocales, Chlorophyta) [J]. *J. Plant Physiol.* 1987, **131**: 479—487

[14] Wynne D et al. The relationship between nutrient status and chemical composition of *Peridinium cinctum* during the bloom in Lake Kinneret [J]. *J. Plankton Res.* 1982, **4**: 125—136

[15] Hertzig R, Dubinsky Z, Berman T. Breakdown of *Peridinium* bloom in Lake Kinneret [A]. *In*: Shuval H I. (Ed) Development in Arid Zone Ecology and Environment Quality [M]. Balaban ISS, Philadephia, PA, 1981, 179—185

[16] Dubinsky Z, Polna M. Pigment composition during a *Peridinium* bloom in Lake Kinneret (Israel) [J]. *Hydrobiologia*, 1976, **51**: 239—243

[17] Hager H. The reverse, light-induced conversions of xanthophylls in the chloroplast [A]. *In*: Czygan, F C.

(Ed) *Pigments in Plants* (2nd edition) [M]. *Fischer*, Stuttgart, 1980, 57—79

[18] Yacobi Y Z, mantoura R F C, Liewellyn C A. The distribution of chlorophylls, carotenoids and their breakdown products in Lake Kinneret (Israel) sediments [J]. *Freshwat. Biol.* 1991, **26**: 1—10

多甲藻对不同光照强度的反应：色素沉着及暗光适应的可能性

刘学君[1] Yosef Z. YACOB[2]

(1. 中国科学院水生生物研究所，武汉 430072；2. The Yigal Allon Kinneret Limnological Laboratory, Israel Oceanographic & Limnological Reaearch Ltd, Tiberias 14102, Israel)

摘要：在 HPLC 色素分析的基础上，研究了多甲藻对不同光照强度(16.6—250μ・$Em^{-2}s^{-1}$)的反应。类胡萝卡素中最重要的多甲藻素和硅甲藻黄素占叶黄素总量的 90%。随光照强度的逐步增加，多甲藻细胞中叶绿素 a 含量呈现明显的下降趋势。结果表明硅甲藻黄素和β胡萝卜素具有光保护作用：1)当多甲藻暴露于较强光下时，硅甲藻黄素 / 叶绿素 a 之比呈现出明显增加的趋势；β胡萝卜素 / 叶绿素 a 之比也显现出明显的增加趋势；2)β胡萝卜素 / 叶绿素 a 之比在有光条件下比在完全黑暗条件下要高；3)硅甲藻黄素 / 叶绿素 a 之比在高辐照度、低辐照度，及由高到低、由低到高辐照度处理时，显示出组间差异。由此可推出色素代谢可能的途径及其湖沼学意义。

关键词：Kinneret 湖；暗光适应；多甲藻；色素沉着