# Acclimation of the photosynthetic apparatus of the endemic Antarctic red macroalga *Palmaria decipiens* to seasonally changing light conditions

# Akklimatisation des Photosyntheseapparates der endemisch antarktischen roten Makroalge *Palmaria decipiens* an saisonal wechselnde Lichtbedingungen

---

Ulrike H. Lüder

**Ber. Polarforsch. Meeresforsch. 469 (2003)**
**ISSN 1618 - 3193**

Ulrike H. Lüder

Institut für Zellbiologie, Biochemie und Biotechnologie
Universität Bremen
Postfach 33 04 40
28334 Bremen

Alfred-Wegener-Institut für Polar und Meeresforschung
in der Helmholtz-Gemeinschaft
Am Handelshafen 12
27570 Bremerhaven

Die vorliegende Arbeit ist die inhaltlich unveränderte Fassung einer kumulativen Dissertation, die bei PD. Dr. Jürgen Knoetzel in der Arbeitsgruppe „Biologie mit dem Schwerpunkt Biochemie und Stoffwechselphysiologie“ von Prof. Dr. L. Horst Grimme am Institut für Zellbiologie, Biochemie und Biotechnologie der Universität Bremen und bei Prof. Dr. Christian Wiencke in der Projektgruppe „Solare UV-Strahlung“ am Alfred-Wegener-Institut angefertigt und Januar 2002 dem Fachbereich 2 (Biologie/Chemie) der Universität Bremen vorgelegt wurde.

## TABLE OF CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| APC | allophycocyanin |
| APB | allophycocyanin B |
| Car | carotinoid |
| Chl *a* | chlorophyll *a* |
| ETR | relative electron transport rate |
| $ETR_{max}$ | maximal ETR |
| $F_m$ | maximal chlorophyll fluorescence of PSII after dark incubation |
| $F_m'$ | maximal chlorophyll fluorescence of PSII after light incubation |
| $F_o$ | minimal chlorophyll fluorescence of PSII after dark incubation |
| $F_0'$ | minimal chlorophyll fluorescence of PSII after light incubation |
| $F_s$ | steady state chlorophyll fluorescence of PSII after light incubation |
| $F_v$ | variable chlorophyll fluorescence of PSII after dark incubation, ($F_v = F_m - F_o$) |
| $F_v/F_m$ | optimal quantum yield of PSII in dark-acclimated state |
| $\Delta F$ | difference between $F_m'$ and $F_s$ |
| $\Delta F/F_m'$ | effective quantum yield of PSII in light-acclimated state |
| FNR | ferredoxin $NADP^+$ oxidoreductase |
| FW | fresh weight |
| $L_C$ | core linker |
| $L_{CM}$ | core membrane linker |
| LHCI | light-harvesting antennae of PSI |
| $L_R$ | rod linker |
| $L_{RC}$ | rod core linker |
| PAM | pulse-amplitude modulated fluorometer |
| PBS | phycobilisome(s) |
| $PB_{tot}$ | total phycobiliproteins (APC+PC+PE) |
| PBS | phycobilisomes |
| PC | phycocyanin |
| PCB | phycocyanobilin |
| PE | phycoerythrin |
| PEB | phycoerythrobilin |
| PFD | photon flux density of actinic irradiance |
| PSI | photosystem I |

| | |
|---|---|
| PSII | photosystem II |
| PUB | phycourobilin |
| PVB | phycoviolobilin |
| RCI | reaction center of PSI |
| RCII | reaction center of PSII |
| SD | standard deviation |

## SUMMARY

*Palmaria decipiens* is an Antarctic endemic and one of the most common red macroalgae in the sublittoral. In this study the influence of the seasonally strongly changing daylengths and of exposure to darkness simulating winter sea-ice cover, on the photosynthetic apparatus of *P. decipiens* was studied in long-term culture experiments. The phycobilisomes, the main light harvesting antennae of red algae consisting of phycobiliproteins, were isolated and characterised (1). Their dynamics in response to the seasonally changing daylengths (2) and to dark exposure (3) was investigated and related to phycobiliprotein tissue contents and to the photosynthetic performance.

1. At first the method of isolation of phycobilisomes was established and the general structure of phycobilisomes of *P. decipiens* studied. The phycobilisomes from *P. decipiens* were isolated on discontinuous sucrose gradients as two discrete bands and not in one as expected. To exclude methodical faults, phycobilisomes from the temperate *Palmaria palmata* and the unicellular red algae *Porphyridium cruentum* and *Rhodella violacea* were isolated as well. In *P. palmata* the phycobilisomes were also separated in two discrete bands, whereas the phycobilisomes from *Porphyridium* and *Rhodella* were found in one band.

The double banded phycobilisomes ($PBS_{up}$ and $PBS_{low}$) from *P. decipiens* were characterised by absorption and fluorescence spectroscopy. Their PE-, PC- and APC-trimers or rather hexamers, inclusive their associated linkers, were isolated by native PAGE and were also characterised by absorption and fluorescence spectroscopy. The α- and β-subunits of PE, PC and APC as well as the associated linker polypeptides and γ-subunits were identified by SDS-PAGE, and their apparent molecular masses were calculated by densitometric analysis. The hemiellipsiodal phycobilisome structure was shown by negative staining and electron microscopy. The phycobiliproteins RIII-phycoerythrin, RI-phycocyanin and allophycocyanin were identified. The $PBS_{up}$ and $PBS_{low}$ showed no significant differences in their absorption spectra and phycobiliprotein ratios, although $PBS_{low}$ seem to be somewhat smaller. Differences were found in their low molecular mass subunit complexes, which are assumed to be r-phycoerythrin. The polypeptide pattern of the $PBS_{up}$ and $PBS_{low}$ showed no differences in the molecular masses of their subunits and linker polypeptides, but in their percentage distribution. The results suggest that the $PBS_{low}$ is a closer packed and $PBS_{up}$ a little more loosely aggregated hemiellipsiodal phycobilisome form.

Furthermore, three coloured γ-subunits ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) and three hexameric PE-subunit complexes ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$) with different absorption characteristics around 544.5 nm and with a red shift in their absorption maxima were identified, both in $PBS_{up}$ and $PBS_{low}$. Moreover, a red shift in their fluorescence emission maxima was detected, which probably improves the energy transfer downhill the rod. The $\gamma^{33.5}$-subunit and its associated PE-hexamer ($(\alpha\beta)_6\gamma^{33.5}$) shows a further fluorescence maximum at 595 nm, which also enhances the energy transfer downhill the rod and makes a coupling of PE directly to APC most probable.

2. The influence of seasonally fluctuating Antarctic daylength on the photosynthetic apparatus of *P. decipiens* was studied in a long-term culture experiment, mimicking the Antarctic year. Over one year *P. decipiens* was cultivated under weekly changing daylengths (under constant irradiance), simulating the seasonal variations of daylength, present at the collecting site on King George Island, South Shetland Islands, Antarctica varying between 5 h in winter and 20 h in summer. The maximal photosynthetic performance ($ETR_{max}$) and the optimal quantum yield ($F_v/F_m$) were measured by *in vivo* chlorophyll fluorescence in monthly intervals. The phycobiliprotein and chlorophyll *a* tissue contents were quantified. Phycobilisomes were isolated monthly and changes in their phycobiliprotein and polypeptide composition were determined.

In Antarctic summer, $ETR_{max}$, $F_v/F_m$ and pigment tissue contents showed its lowest values, probably to avoid photodamage caused by excess light energy. In contrast, $F_v/F_m$ was constantly high during mid autumn, winter and spring, indicating an intact photosynthetic apparatus. $ETR_{max}$ and pigment tissue contents increased with the beginning of autumn (from April/May onwards) more or less in parallel continuously to its highest values at the beginning of Antarctic spring (September/October). For this time, a positive correlation between pigments and $ETR_{max}$ was found.

$PBS_{up}$ and $PBS_{low}$ varied in their size during the entire Antarctic year. 'Small' phycobilisomes (lowest PE:APC and PE:PC ratios) were found at the end of Antarctic summer in April. With the begin of autumn (from April/May) a slight and from August (Antarctic winter) on a clear continuous increase in phycobilisome size was observed, resulting in 'large' phycobilisomes in Antarctic spring (November), both in $PBS_{up}$ and $PBS_{low}$.

The phycobilisome size was modulated by changing only the PE part within the rods, whereas the PC part seems to be unaltered, indicating no alteration in the rod number. The rod length seemed to be altered primarily by coupling and uncoupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. The predominant $\gamma^{37.3}$-subunit varied only little during the

entire year, but a further coupling of $\gamma^{37.3}$-subunits and their associated PE-Hexamers was assumed in autumn (August-June). In contrast, the $\gamma^{40.3}$-subunit did not vary with the seasons.

The $\gamma^{33.5}$-subunit is located at the periphery of the rods, furthermore we propose a rod only consisting of $\gamma^{33.5}$-subunits and their associated PE hexamers. The predominant $\gamma^{37.3}$-subunit is located at the innermost PE part of the rod. The $\gamma^{40.3}$-subunit is suspected to be at a peripheral position, close to the thylakoid membrane. The seasonal variation in the proportions of the two core membrane linkers $L_{CM}^{85.0}$ and $L_{CM}^{75.4}$ indicates an acclimation of the phycobilisome core, both in $PBS_{up}$ and $PBS_{low}$.

$PBS_{up}$ and $PBS_{low}$ varied also in their amount during the entire Antarctic year. The total phycobilisome number seems to increase with the begin of Antarctic autumn (from April/May onwards) more or less continuously until Antarctic spring (October/November), related from the seasonal changes in the APC tissue content.

The relative proportion of $PBS_{up}$ and $PBS_{low}$ clearly changed during the Antarctic year. $PBS_{low}$ was nearly negligible in February (Antarctic summer). The appearance of $PBS_{up}$ and $PBS_{low}$ can be seen in relationship to the seasonal changes in the phycobiliprotein tissue content. A conversion of $PBS_{up}$ into $PBS_{low}$ is suggested. Different physiological functions to $PBS_{up}$ and $PBS_{low}$ are discussed.

3. The influence of extended winter sea-ice cover, simulated by a dark period (from 2nd April to 1st October), and of subsequent re-exposure to light under seasonally fluctuating Antarctic daylengths was investigated in a parallel long-term experiment in monthly intervals. In October, the acclimation to light was investigated in more detail and subsamples were taken in short time intervals during the first 4 weeks after re-illumination of dark-exposed plants.

During the first half of the dark period, a slight increase in pigment contents was observed. After four months in darkness a degradation of the photosynthetic apparatus was evident by a strong decrease in $ETR_{max}$ and $F_v/F_m$ as well as in the phycobiliprotein tissue contents. At the end of the dark period, *P. decipiens* lost its ability to photosynthesise. The time course of re-illumination showed that *P. decipiens* started by 24h after re-illumination to accumulate Chl *a* and to photosynthesise. The phycobiliprotein accumulation started after a lag time of about seven days. *P. decipiens* reached $ETR_{max}$ values, comparable to the values before darkness, seven days after re-illumination and maximal values after 30 days of re-illumination. For this time (October), a positive correlation between pigments and $F_v/F_m$ and between pigments and $ETR_{max}$ was found, but above a certain level of phycobiliproteins, a further increase led to no further

rise in $F_v/F_m$ and $ETR_{max}$. In summer, *P. decipiens* reduced its photosynthetic performance and pigment contents, like previously observed.

$PBS_{up}$ and $PBS_{low}$ were found in March and April before the dark period. After one month of darkness, $PBS_{low}$ had still constant PE:APC, PE:PC and PC:APC ratios, indicating no alteration in the phycobilisome size. From the second month of darkness onwards, $PBS_{low}$ completely disappeared. Since no decrease in the phycobiliprotein tissue contents was found, we assume a spontaneously disruption of $PBS_{low}$ or a conversion of $PBS_{low}$ into $PBS_{up}$. The amount of remaining $PBS_{up}$ started to decrease after four months of darkness. The size of $PBS_{up}$ seemed to remain unaltered over the first five months of darkness. In the last month of darkness a degradation of the remaining $PBS_{up}$ occurred by uncoupling of hole rods. The rod loss was induced by the loss of the rod core linker $L_{RC}^{30.6PC}$ and the rod linker $L_R^{38.9PC}$, furthermore the $\gamma^{40.3}$-subunit was reduced. The $\gamma^{33.5}$-subunits did not vary during darkness. The remaining $\gamma^{37.3}$-subunit became much more dominant in the last two months of darkness.

After one months of re-illumination, $PBS_{up}$ was fully recovered. Moreover the phycobilisome size increased strongly by coupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. $PBS_{low}$ appeared again and also increased in size by coupling of the $\gamma^{33.5}$-subunits and its associated PE hexamer, but the coupling of $L_{RC}^{30.6PC}$ and of $L_R^{38.9PC}$ and their associated PC trimers was not recovered before February. This suggests that there are rods only consisting of PE coupled directly via the $\gamma^{33.5}$-subunit to APC. However, in the Antarctic summer months (January-March), the amount of the $\gamma^{33.5}$-subunit decreased, indicating a reduction in phycobilisome size by uncoupling of some PE, both in $PBS_{up}$ and $PBS_{low}$.

The conclusion of this study is, that *P. decipiens* is efficiently adapted to the seasonally short period of favourable light conditions in the field. The alga starts in April to increase the number and the size of $PBS_{up}$ and $PBS_{low}$ and in July to increase the amount of the reaction centres or even the size of LHCI complexes more or less continuously during the entire autumn, winter and spring. Both mechanisms leads to a parallel increase in the photosynthetic performance, which is subsequently maximal in Antarctic spring. This is the time, when the water is very clear and the sunlight penetrates deep into the water column, after break up of sea ice and before the water becomes turbid due to plankton blooms and glacial melt water. In this short light window, *P. decipiens* has to produce biomass and reserves. During Antarctic summer, *P. decipiens* reduces its photosynthetic apparatus to a minimum. These results confirm with the life strategy of *P. decipiens* as season anticipator.

*P. decipiens* is even very well adapted to a prolonged dark period experienced in the field. In the first three months of darkness, the photosynthetic apparatus is more or less unaffected. In the last three months of darkness a clear disruption of the photosynthetic apparatus occurred, indicated by a strong reduction in optimal quantum yield, photosynthetic performance, phycobiliprotein contents and in the amount of $PBS_{up}$. A degradation of the remaining $PBS_{up}$ and probably of reaction centres and of the LHCI complexes started in the last two months of darkness. After six months of darkness *P. decipiens* looses its ability to photosynthesise. Nevertheless, *P. decipiens* starts already 24h after re-illumination, to photosynthesise, which would assume a quick repair mechanisms. The recovery of the photosynthetic performance is accelerated by the accumulation of pigments. Likewise, *P. decipiens* reaches maximal values in November, in Antarctic spring under the best light conditions in the field, like previously discussed.

In summary, *P. decipiens* is highly adapted to seasonally changing light conditions and to prolonged darkness. The seasonal changes in pigments and photosynthesis seems to follow a fixed seasonal pattern and suggest the hypothesis of a photoperiodic control of pigment synthesis and may be of photosynthesis triggered by daylength.

The phycobilisomes are extremely variable and showed several mechanisms to enhance the energy transfer downhill the rod. Moreover, new strategies in the modification of the phycobilisomes during changing light conditions were found in *P. decipiens*. These results demonstrate the strong need to investigate phycobilisomes in detail, and not only phycobiliprotein tissue contents of Antarctic macroalgae and generally of the more advanced red algae belonging to the Florideophyceae.

# ZUSAMMENFASSUNG

*Palmaria decipiens* ist eine endemisch antarktische Rotalge und eine der meist verbreiteten Makroalgen des südlichen Ozeans. In dieser Arbeit wurde der Einfluß der saisonal stark schwankenden Tageslänge und der Einfluß von die Meereisbedeckung simulierender winterlicher Dunkelheit auf den Photosyntheseapparat von *P. decipiens* in Langzeit-Kulturexperimenten untersucht. Phycobilisomen, die aus Phycobiliproteinen bestehenden Hauptlichtsammelantennen der Rotalgen wurden isoliert und charakterisiert (1). Ihre Dynamik unter dem Einfluß der saisonal schwankenden Tageslänge (2) und einer Dunkelexposition (3) wurden untersucht und in Beziehung zum Phycobiliproteingehalt und zur photosynthetischen Aktivität der Algen gebracht.

1. Zunächst wurden die Methoden zur Isolierung von Phycobilisomen etabliert und die generelle Struktur der Phycobilisomen von *P. decipiens* untersucht. Sie wurden auf diskontinuierlichen Zuckergradienten als zwei diskrete Banden und nicht wie erwartet als eine Bande isoliert. Um methodische Fehler auszuschließen, wurden außerdem die kaltgemäßigte *Palmaria palmata* und die einzelligen Rotalgen *Porphyridium cruentum* und *Rhodella violacea* untersucht. Aus *P. palmata* wurden die Phycobilisomen ebenfalls in zwei diskreten Banden isoliert, wohingegen die Phycobilisomen aus *Porphyridium* und *Rhodella* nur in einer Bande erschienen.

Die doppelbandigen Phycobilisomen ($PBS_{up}$ und $PBS_{low}$) von *P. decipiens* wurden anhand ihrer Absorptions- und Fluoreszenzeigenschaften charakterisiert. Ihre PE-, PC- und APC-Trimere bzw. Hexamere, inklusive ihrer assoziierten Linker, wurden durch native PAGE isoliert und ebenfalls mittels Absorptions- und Fluoreszenzspektroskopie charakterisiert. Die $\alpha$- und $\beta$- Untereinheiten von PE, PC und APC sowie die assoziierten Linker-Polypeptide und $\gamma$-Untereinheiten wurden mittels SDS-PAGE identifiziert, und ihre apparenten Molekularmassen wurden mittels densitometrischer Analysen berechnet. Die hemiellipsoidale Struktur der Phycobilisomen wurde durch Negativfärbung im Elektronenmikroskop nachgewiesen. Die Phycobiliproteine RIII-Phycoerythrin, RI-Phycocyanin und Allophycocyanin wurden identifiziert. $PBS_{up}$ und $PBS_{low}$ zeigten keine Unterschiede in ihren Absorptionsspektren und Phycobiliprotein-Verhältnissen, obwohl $PBS_{low}$ etwas kleiner zu sein schienen. Unterschiede wurden in ihrem niedermolekularer PE-Untereinheiten-Komplex, welcher anscheinend r-Phycoerythrin darstellt, gefunden. Die Polypeptid-Muster von $PBS_{up}$ und $PBS_{low}$ zeigten keine Unterschiede in den Molekularmassen ihrer Untereinheiten und

Linker-Polypeptide, aber in ihrer prozentualen Verteilung. Die Ergebnisse zeigen, daß $PBS_{low}$ eine etwas dichter und $PBS_{up}$ eine etwas lockerer gepackte hemiellipsiodale Phycobilisomform ist.

Sowohl in $PBS_{up}$ als auch in $PBS_{low}$ wurden drei farbige γ-Untereinheiten ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) und drei hexamere PE-Untereinheiten-Komplexe ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$) gefunden. Letztere unterscheiden sich in ihren Absorptionseigenschaften bei 544.5 nm und zeigen eine Rotverschiebung in ihren Absorptionsmaxima. Des Weiteren wurde eine Rotverschiebung in ihren Fluoreszenzemisionsmaxima entdeckt, die wahrscheinlich den Energietransfer innerhalb der Stäbchen der Phycobilisomen verbessert. Die $\gamma^{33.5}$-Untereinheit mit ihrem assoziierten PE-Hexamer ($(\alpha\beta)_6\gamma^{33.5}$) zeigt ein weiteres Fluoreszenzmaximum bei 595 nm, welches ebenfalls den Energietransport entlang der Stäbchen förderlich ist und sogar eine Kopplung direkt an APC wahrscheinlich macht.

2. Der Einfluß der saisonal schwankenden antarktischen Tageslänge auf den Photosyntheseapparat von *P. decipiens* wurde in einem Langzeit-Kulturexperiment, das den antarktischen Jahresgang simuliert, untersucht. Über ein Jahr wurde *P. decipiens* unter wöchentlich wechselnden Tageslängen zwischen 5 h im Winter und 20 h im Sommer (bei konstanter Lichtintensität), entsprechend den saisonal wechselnden Tageslängen am Sammelort bei King George Island, South Shetland Islands, Antarktis, kultiviert. In monatlichen Intervallen wurden die maximale photosynthetische Aktivität ($ETR_{max}$) und die optimale Quantenausbeute ($F_v/F_m$) mittels *in vivo* Chlorophyll-Fluoreszenz gemessen. Zudem wurden die Phycobiliprotein- und Chlorophyll *a*-Gehalte der Algen quantifiziert. Phycobilisomen wurden ebenfalls monatlich isoliert und Änderungen in der Zusammensetzung ihrer Phycobiliproteine und Polypeptide wurden untersucht.

Im antarktischen Sommer wies *P. decipiens* die niedrigsten Werte für $ETR_{max}$, $F_v/F_m$ und für die Pigmentgehalte der Algen auf, wahrscheinlich um eine Photoschädigung durch überschüssige Lichtenergie zu vermeiden. Hingegen war $F_v/F_m$ konstant hoch während Mitte-Herbst, Winter und Frühjahr, was für einen intakten Photosyntheseapparat spricht. $ETR_{max}$ und die Pigmentgehalte der Algen stiegen mit Herbstbeginn (von April/Mai) kontinuierlich über die Wintermonate zu ihren höchsten Werten im antarktischen Frühlingsbeginn (September/Oktober) an. Für diesen Zeitraum wurde eine positive Korrelation zwischen Pigmentgehalt der Algen und $ETR_{max}$ gefunden.

$PBS_{up}$ und $PBS_{low}$ variierten in ihrer Größe während des antarktischen Jahres. ‚Kleine' Phycobilisomen (niedrige PE:APC- und PE:PC-Verhältnisse) wurden nach dem antarktischen Sommer im April gefunden. Mit Herbstbeginn (von April/Mai) erfolgte ein

leichter und von August (antarktischer Winter) an ein deutlicher kontinuierlicher Anstieg in der Phycobilisomengröße, so daß im antarktischen Frühling (November) ‚große' Phycobilisomen vorlagen, sowohl in $PBS_{up}$ als auch in $PBS_{low}$.
Die Größe der Phycobilisomen wurde moduliert durch Variation des PE-Anteils, wohingegen der PC-Anteil unverändert blieb, was für eine Konstanz in der Anzahl der Stäbchen spricht. Die Länge der Stäbchen veränderte sich in erster Linie durch das An- und Abkoppeln von $\gamma^{33.5}$-Untereinheiten und ihren assoziierten PE-Hexameren. Das Vorkommen der dominierenden $\gamma^{37.3}$-Untereinheit variierte nur geringfügig während des antarktischen Jahres. Jedoch wurde eine zusätzliche Ankopplung von $\gamma^{37.3}$-Untereinheiten und ihren assoziierten PE-Hexameren im Herbst (August-Juni) beobachtet. Hingegen blieb das Vorkommen der $\gamma^{40.3}$-Untereinheit mit den Jahreszeiten unverändert.
Die $\gamma^{33.5}$-Untereinheit ist an der Peripherie der Stäbchen lokalisiert. Zudem gibt es wahrscheinlich Stäbchen, die ausschließlich aus $\gamma^{33.5}$-Untereinheiten und ihren assoziierten PE-Hexameren zusammengesetzt sind. Die dominierende $\gamma^{37.3}$-Untereinheit ist am innersten PE-Anteil der Stäbchen lokalisiert. Die $\gamma^{40.3}$-Untereinheit wird an einer peripheren Position vermutet, wahrscheinlich nahe der Thylakoidmembran. Die jahreszeitliche Variation in den Proportionen der zwei Kernmembran-Linker $L_{CM}^{85.0}$ und $L_{CM}^{75.4}$ deutet auf eine Akklimatisation des Kerns der Phycobilisomen hin, sowohl in $PBS_{up}$ als auch in $PBS_{low}$.

$PBS_{up}$ und $PBS_{low}$ variierten zudem in ihrer Anzahl während des antarktischen Jahres. Die Menge der Phycobilisomen stieg mit dem antarktischen Herbstanfang (von April/Mai) mehr oder weniger kontinuierlich bis zum antarktischen Frühling (Oktober/November) an, wie man aus der Veränderung der APC-Gehalte der Algen entnehmen kann. Dabei änderten sich die relativen Verhältnisse von $PBS_{up}$ und $PBS_{low}$ deutlich während des antarktischen Jahres. $PBS_{low}$ konnte im Februar (antarktischer Sommer) kaum nachgewiesen werden. Das Auftreten von $PBS_{up}$ und $PBS_{low}$ konnte in Beziehung zu den saisonalen Veränderungen der Phycobiliproteingehalte der Algen gebracht werden. Eine Konversion von $PBS_{up}$ in $PBS_{low}$ wurde vorgeschlagen und unterschiedliche physiologische Funktionen von $PBS_{up}$ und $PBS_{low}$ wurden diskutiert.

3. Der Einfluß einer ausgedehnten winterlichen Meereisbedeckung, simuliert durch eine Dunkelperiode (vom 2. April bis 1. Oktober), und einer anschließenden Lichtexposition mit saisonal schwankenden antarktischen Tageslängen auf den Photosyntheseapparat von *P. decipiens* wurde in einem parallel laufenden Langzeit-Kulturexperiment in monatlichen Intervallen untersucht. Im Oktober wurde die

Akklimatisation an Licht detailliert beobachtet, und Unterproben wurden in Kurzzeit-Intervallen während den ersten vier Wochen nach der Wiederbelichtung genommen.

In der ersten Hälfte der Dunkelphase wurde ein leichter Anstieg in den Pigmentgehalten der Algen beobachtet. Nach viermonatiger Dunkelheit erfolgte eine Degradation des Photosyntheseapparates, die sich in einem starken Abfall in $ETR_{max}$, $F_v/F_m$ und in den Phycobiliproteingehalten der Algen äußerte. Am Ende der Dunkelperiode verlor *P. decipiens* ihre Möglichkeit zu photosynthetisieren. Innerhalb von 24 h nach Wiederbelichtung erhöhte sich der Gehalt an Chl *a*, und *P. decipiens* begann mit der Photosynthese. Nach einer lag-Phase von etwa sieben Tagen erhöhte sich der Gehalt an Phycobiliproteinen. *P. decipiens* erreichte zu diesem Zeitpunkt $ETR_{max}$-Werte, die den Werten zu Beginn der Dunkelperiode entsprachen. Maximale Werte wurden 30 Tage nach Wiederbelichtung erreicht. Für diesen Zeitraum (Oktober) wurde eine positive Korrelation zwischen Pigmentgehalt der Algen und $F_v/F_m$ sowie zwischen Pigmentgehalt der Algen und $ETR_{max}$ gefunden, wobei jedoch ab einem bestimmten Level an Phycobiliproteinen eine weitere Erhöhung zu keinem weiteren Anstieg in $F_v/F_m$ und $ETR_{max}$ führte. Im Sommer verminderte sich die Photosyntheseaktivität von *P. decipiens*. Außerdem verminderten sich die Pigmentgehalte wieder, was beides bereits im Langzeit-Experiment ohne Dunkelexposition beobachtet wurde.

$PBS_{up}$ und $PBS_{low}$ waren in den Monaten vor der Dunkelperiode präsent. Nach einmonatiger Dunkelheit zeigte $PBS_{low}$ immer noch konstante PE:APC, PE:PC und PC:APC Verhältnisse, was auf keine Veränderung in der Größe von $PBS_{low}$ deutet. Nach zweimonatiger Dunkelheit verschwand $PBS_{low}$ komplett. Da keine Abnahme in den Phycobiliproteingehalten der Algen gemessen wurde, ist ein spontaner Zerfall von $PBS_{low}$ aber auch eine Konversion von $PBS_{low}$ in $PBS_{up}$ anzunehmen. Die Anzahl der verbliebenen $PBS_{up}$ begann erst nach viermonatiger Dunkelheit abzunehmen. Die Größe von $PBS_{up}$ scheint auch während der ersten fünf Monate in Dunkelheit unverändert zu bleiben. Im letzten Monat in Dunkelheit wurde eine Degradation der verbliebenen $PBS_{up}$ durch eine Abkopplung ganzer Stäbchen beobachtet. Der Verlust der Stäbchen wurde durch den Verlust des Stäbchen-Kern-Linkers $L_{RC}^{30.6PC}$ und des Stäbchen-Linkers $L_R^{38.9PC}$ induziert. Zudem wurde die Abundanz der $\gamma^{40.3}$-Untereinheit reduziert. Die $\gamma^{33.5}$-Untereinheit änderte sich während der Dunkelperiode nicht. Die verbliebene $\gamma^{37.3}$-Untereinheit wurde noch dominanter in den letzten zwei Monaten in Dunkelheit.

Nach einmonatiger Wiederbelichtung war $PBS_{up}$ vollständig wieder hergestellt. Überdies stieg die Größe von $PBS_{up}$ extrem durch Ankopplung der $\gamma^{33.5}$-Untereinheit und ihrem assoziierten PE-Hexamer an. $PBS_{low}$ erschien auch wieder und seine Größe erhöhte sich ebenfalls durch Ankopplung der $\gamma^{33.5}$-Unterheit und ihrem assoziierten PE-Hexamer.

Die Kopplung von $L_{RC}^{30.6PC}$ und $L_{R}^{38.9PC}$ und ihren assoziierten PC-Trimeren wurde nicht vor Februar wiederhergestellt. Dies läßt vermuten, daß es Stäbchen gibt, die nur aus PE-Hexameren zusammengesetzt sind, die über die $\gamma^{33.5}$-Untereinheit direkt an APC gekoppelt sind. In den antarktischen Sommermonaten Januar-März nimmt die Abundanz der $\gamma^{33.5}$-Unterheit wieder ab, was zu einer Reduzierung der Größe der Phycobilisomen durch Abkopplung von PE führte, sowohl in $PBS_{up}$ als auch in $PBS_{low}$.

Die Schlußfolgerung dieser Arbeit ist, daß *P. decipiens* effizient an die jahreszeitlich kurzzeitig günstigen Lichtbedingungen im Feld adaptiert ist. Die Alge beginnt im April die Anzahl und Größe von $PBS_{up}$ und $PBS_{low}$ und im Juli die Anzahl der Reaktionszentren oder auch die Größe von LHCI-Komplexen während den Herbst-, Winter- und Frühjahrsmonaten mehr oder wenig kontinuierlich zu steigern. Beides führt zu einem parallelen Anstieg in der photosynthetischen Aktivität, die folglich maximal im Antarktischen Frühling ist. Zu dieser Jahreszeit bricht das Meereis auf, das Wasser ist sehr klar und das Sonnenlicht kann tief die Wassersäule durchdringen. Dieses kleine „Lichtfenster" nutzt *P. decipiens* aus, um Biomasse und Reserven aufzubauen. Während des antarktischen Sommers, wenn das Wasser durch Planktonblüten und durch Einträge von Gletschern wieder trübe wird, reduziert *P. decipiens* den Photosyntheseapparat auf ein Minimum. Diese Ergebnisse bekräftigen die Lebensstrategie von *P. decipiens* als „season anticipator".

*P. decipiens* ist auch sehr gut an eine ausgedehnte Dunkelperiode, wie sie im Felde vorkommen kann, angepaßt. In den ersten drei Monaten in Dunkelheit ist der Photosyntheseapparat mehr oder weniger unbeeinflußt. Mit dem vierten Monat in Dunkelheit beginnt ein klarer Abbau des Photosyntheseapparates, was sich in eine starke Reduzierung der optimalen Quantenausbeute, der photosynthetischen Aktivität, der Phycobiliproteingehalte der Algen und der Anzahl der Phycobilisomen äußerte. Eine Degradation der verbliebenen $PBS_{up}$ und wahrscheinlich auch der Reaktionszentren und der LHCI-Komplexe begann nach fünfmonatiger Dunkelheit. Nach sechsmonatiger Dunkelheit verlor *P. decipiens* die Möglichkeit zur Photosynthese. Trotzdessen beginnt *P. decipiens* bereits 24 h nach Wiederbelichtung zu photosynthetisieren, ein Indiz für einen schnellen Reparaturmechanismus. Die Erholung der photosynthetischen Performanz wird beschleunigt durch eine Akkumulation der Pigmente. Somit erreicht *P. decipiens* maximal Werte im November, im antarktischen Frühjahr, wenn kurzzeitig günstigste Lichtbedingungen im Feld vorliegen, wie bereits zuvor diskutiert wurde.

Zusammenfassend kann gesagt werden, daß *P. decipiens* sich an die saisonal wechselnde Lichtbedingungen und an eine ausgedehnte Dunkelperiode gut anpassen kann. Die saisonalen Änderungen der Pigmente und der Photosynthese scheinen einem festen saisonalen Muster zu unterliegen und erlauben die Hypothese einer photoperiodisch kontrollierten Pigmentsynthese und eventuell auch Photosynthese, gesteuert durch die Tageslänge.

Die Phycobilisomen sind extrem variabel und zeigen mehrere Mechanismen den Energietransfer entlang der Stäbchen zu verbessern. Zudem wurden in *P. decipiens* neue Strategien bei der Anpassung der Phycobilisome an veränderte Lichtbedingungen gefunden. Diese Ergebnisse zeigen die starke Notwendigkeit, Phycobilisome im Detail zu studieren und nicht nur den Phycobiliproteingehalt der antarktischen Makroalgen und generell der höher entwickelten roten Algen, den Florideophyceaen, zu untersuchen.

# 1. INTRODUCTION

## 1.1. The life strategy of Antarctic macroalgae

The Antarctic marine benthic algal flora comprises at least 119 species (Wiencke and Clayton 2002). Their distribution is circumpolar, but nearly half of them are restricted to the Antarctic Peninsula and its surrounding islands (South Shetlands Islands, South Orkneys Islands, see Fig. 9) (Heywood and Whittaker 1984). 33% of the Antarctic species recorded today are endemic (Wiencke and Clayton 2002). This is a very high percentage, compared to the Arctic, where only very few endemic species are recorded (Lüning 1990, Dunton 1992). In the last decade, the ecophysiology, life history and biogeography of Antarctic macroalgae has been studied intensively as recently reviewed (Wiencke and Kirst 1995, Wiencke 1996, Wiencke and Clayton 2002).

In the Antarctic, benthic macroalgae are restricted almost exclusively to the sublittoral (Lamb and Zimmermann 1977, Wiencke and Clayton 2002) and exposed to constant water temperatures (-1.8 to +2.0°C) and high nutrient concentrations over the entire year (Clarke et al. 1988, Drew and Hastings 1992, Klöser et al. 1993). Therefore, the seasonal development of Antarctic macroalgae depends mainly on the seasonal variation of light conditions, especially of the daylength. The underwater light climate is furthermore affected by ice cover in winter. Especially if the ice is covered with snow, dim light or complete darkness may prevail in the sublittoral for up to ten months per year (Zielinski 1990, Miller and Pearse 1991, Drew and Hastings 1992, Klöser et al. 1993). In summer, the water commonly becomes turbid, due to plankton blooms and glacial melt water (Klöser et al. 1993). Consequently, optimal light conditions for sublittoral macroalgae are present only for a short time in the Antarctic spring, shortly after break up of the sea ice. At this time the water is very clear and the sunlight penetrates deeply into the water column (Klöser et al. 1993). How do sublittoral Antarctic macroalgae acclimate to these seasonal changes in light, and how do they cope with extended periods of winter sea-ice cover ?

The isolation and cultivation of a large number of Antarctic species was a great advance in the investigation of Antarctic macroalgae (Clayton and Wiencke 1986, Wiencke 1988). Seasonal growth and reproduction patterns of Antarctic macroalgae could be simulated in long-term culture studies by mimicking the seasonal variations of daylength, present at the collecting site on King George Island, South Shetland Islands,

Antarctica varying between 5 h in winter and 20 h in summer (Wiencke 1990a, Wiencke 1990b; Publ. 3, Fig. 1). Seasonal formation of gametes/spores and the induction of seasonal growth were monitored much more closely than possible in the field. The results of these studies complement the available fragmentary field observations and indicate that the phenology of Antarctic macroalgae can be controlled in the laboratory (Wiencke 1996).

In these long-term culture studies it has been demonstrated, that growth of Antarctic macroalgae follows two different strategies in order to cope with the strong seasonality of the light regime. One group, mainly endemics such as the species studied here, *Palmaria decipiens*, begins to grow and reproduce already under short day conditions in late winter-spring, even under sea ice. The second group, mainly Antarctic-cold temperate species, starts growth later coinciding with favourable light conditions in spring and summer (Wiencke 1990a, Wiencke 1990b, Gómez et al. 1995a, Gómez and Wiencke 1997, Weykam et al. 1997). The first group has been classified as "season anticipators"; their annual growth and reproduction appears to be controlled by photoperiodisms and by circannual rhythms, triggered or synchronised by daylength (Kain 1989, Wiencke 1996). The second group is called "season responders". These species react directly to changing environmental conditions and show an opportunistic life strategy. In addition to seasonal growth, the seasonal photosynthetic performance of some Antarctic species has also been studied in long-term culture experiments (Daniel 1992, Weykam and Wiencke 1996, Gómez and Wiencke 1997).

The ecophysiology and life strategy of the endemic Antarctic *Palmaria decipiens* as one of the most common Antarctic 'season anticipators' has been studied in more detail. *P. decipiens* lives in the upper sublittoral (Wiencke and Clayton 2002). It is pseudoperennial and develops new blades during Antarctic late winter/early spring (Wiencke 1990b). Growth starts in July (winter) and is as is the case for photosynthesis, maximal in spring (October/November) (Wiencke 1990b, Weykam and Wiencke 1996). The light requirements for growth and photosynthesis of this species are very low (Wiencke 1990b, Wiencke et al. 1993, Weykam et al. 1996). In a pilot-study, winter sea-ice cover was simulated by interrupting the alteration of Antarctic daylengths with a period of six months of darkness during the winter months (April-October) (Weykam et al. 1997). Even in darkness *P. decipiens* starts to develop new blades in early August; supporting the theory of circannual rhythms for growth, triggered or synchronized by daylength. However, growth rates are low or even negative during darkness, but maximal in spring after re-illumination. Photosynthetic performance, measured as oxygen evolution, is reduced dramatically during darkness, but recovers in spring to maximal

values. The long period of darkness was sustained probably by using floridean starch accumulated in the previous summer.

Very little is known about the main light harvesting antennae of Antarctic red algae, the phycobilisomes, consisting of phycobiliproteins, the main light harvesting pigments in red algae. As shown for numerous temperate red algae and cyanobacteria, the phycobilisome structure and the phycobiliprotein composition are highly variable in the course of the acclimation processes to varying light intensity, light quality and nutrient availability. (Gantt 1990, Grossman et al. 1994, Talarico 1996, Talarico 2000). In *Iridaea cordata* collected under ice and in ice free areas in the Antarctic, discrepancies between phycobiliprotein tissue content and phycobilisome assembly have been detected (Foltran et al. 1996), supporting the interest of this kind of studies. Moreover, a novel phycobiliprotein type has been discovered in the Antarctic red alga *Phyllophora antarctica* (MacColl et al. 1996, MacColl et al. 1999). So, there is a strong need to investigate phycobilisomes of macroalgae, living under such extreme conditions as in the Antarctic. In the following chapter, an overview of the present state of knowledge of phycobilisomes and phycobiliproteins is given.

## 1.2. The phycobilisomes and phycobiliproteins

Phycobilisomes are the main light-harvesting antennae in red algae and in cyanobacteria. Structure, composition, spectral properties and the energy flow of phycobilisomes and phycobiliproteins have extensively been studied, see reviews of Gantt 1990, Holzwarth 1991, Mörschel 1991, Tandeau de Marsac 1991, Grossman et al. 1993, Reuter and Müller 1993, Sidler 1994, Apt et al. 1995, Bald et al. 1996, Talarico 1996, Anderson and Toole 1998, MacColl 1998, van Thor et al. 1998. Most of this studies have been performed on cyanobacteria and unicellular red algae genera such as *Porphyridium* or *Rhodella*. There is only a limited number of studies on macroalgae, mainly on genera such as *Porphyra,* belonging to the red algal class Bangiophyceae. Comparably few studies exist on advanced red macroalgae (Florideophyceae).

Phycobilisomes are structures, attached to the stromal side of the thylakoids, as illustrated in electron micrographs of chloroplasts of *Palmaria decipiens* (Fig. 1) and in the model of a thylakoid membrane from cyanobacteria (Fig. 2). They are constructed of two main structural elements: a core in the centre and several peripheral cylindrical rods. In red algae, the common phycobiliproteins are phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). PE is located at the periphery of the rods, PC at the inner part of

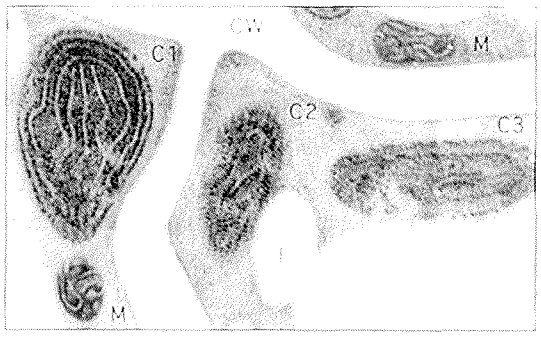


**Fig. 1.** Phycobilisomes in chloroplasts (C1-C3) of *Palmaria decipiens*. The phycobilsomes are seen in face view, attached to the thylakoid membrane in C1. Phycobilisomes are seen in top view, regular arranged in parallel rows on the thylakoid membrane in C2 and C3. Mitochondria (M), floridean starch granule (F), cell wall (CW).

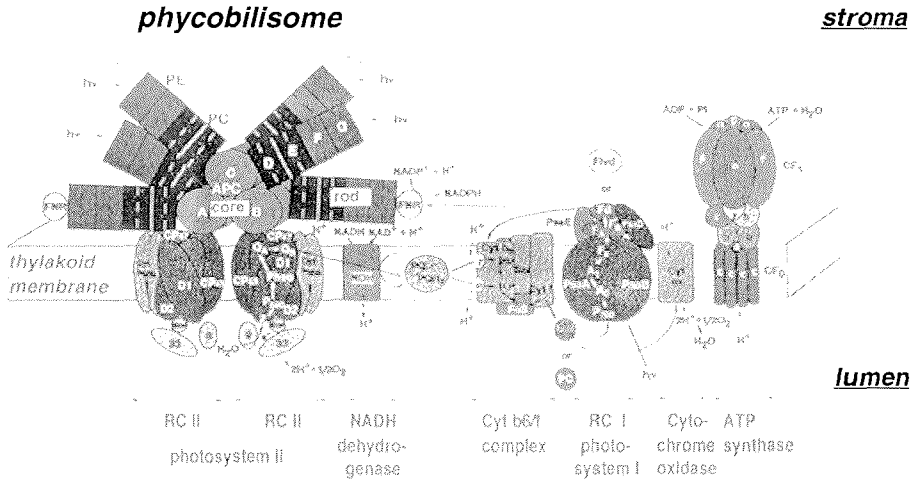


**Fig. 2.** Model of the thylakoid membrane from cyanobacteria and from red algae (with reservations) with the major photosynthetic electron transport components. The water splitting Mn-complex is associated with photosystem II (PSII). PSII is imposed as dimer with two reaction centres (RCII) including the D1 and D2 protein, as well with the intrinsic Chl *a* antennae CP43 and CP47 (CP: chlorophyll protein). Cyanobacteria and red algae do not contain the light-harvesting complex LHC II (Chl *a/b*-carotenoid complex),which is known from higher plants; instead, they have extramembraneous antenna structures, the phycobilisomes (PBS). PBS are constructed of two elements: a central core (A-C) and its surrounding rods (1 rod=D-G). The core contains the phycobiliprotein allophycocyanin (APC), whereas phycoerythrin (PE) is found at the periphery, and phycocyanin (PC) at the basis of the rods. The excitation energy is transferred downhill from the periphery of the rods to the core and than to a special pair Chl *a* of RCII. One PBS is attached to a dimeric RCII. FNR (Ferredoxin-NADP$^+$ Oxidoreductase) might be attached to peripheral rods of PBS to protect the PS I against toxic superoxide radicals formed by oxidation of reduced ferredoxin (Fd) by oxygen. In cyanobacteria, PSI is composed of the reaction centre (RCI). In contrast, red algae have a further PSI specific antenna (LHC I, a Chl *a*-carotenoid complex). (From Sidler 1994, modified)

the rods and APC in the core. Absorbed light energy is transferred from the outer part of the rods, downhill to the final energy transmitter in the core. Phycobilisomes form supramolecular complexes of 5-30 MDa, consisting of phycobiliproteins bound together by linker polypeptides, most of which do not bear chromophores. The phycobiliproteins make up about 80 % and the linker polypeptids about 20 % of the phycobilisome molecular mass.

The phycobilisome structure and the phycobiliprotein composition is very dynamic. The phycobilisome size can be modulated by reducing the rod length or even by the loss of whole rods. A modification of the core composition, changing the energy distribution is also possible. In cyanobacteria, even an exchange of phycobiliprotein classes can occur. At last, the total amount of phycobilisomes is variable. In the following, the phycobilisome structure, phycobiliproteins, linker polypeptides and genes encoding phycobilisome components are described in respect to red algae, but also in consideration of differences to cyanobacteria.

### 1.2.1. Phycobilisome structure

The phycobilisomes were first described as unidentified granules on the stromal side of the photosynthetic lamellae in *Porphyridium cruentum* by Gantt and Conti (1965 and 1966). With the isolation of these granules (Gantt and Conti 1967), it has been prooved that they contain phycobiliproteins and subsequently, they were called phycobilisomes.

Four morphologically different types of phycobilisomes have been described (Wehrmeyer 1983, Mörschel and Rhiel 1987): 1) hemidiscoidal, 2) hemiellipsoidal, 3) bundle-shaped and 4) block-shaped phycobilisomes. The last two types were only found in the cyanobacterium *Gloeobacter violaceus* (Guglielmi et al. 1981) and in the macroalga *Griffithsia pacifica* (Gantt and Lipschultz 1980), respectively, and are not described here.

The best described phycobilisome type is the hemidiscoidal phycobilisome, which was found in most cyanobacteria (Bryant et al. 1979, Mörschel and Rhiel 1987, Ducret et al. 1996) and in some unicellular red algae such as *Rhodella violacea* (Mörschel 1982, Mörschel et al. 1977). They consist of six cylindrical rods (each about 11 nm in diameter and 12-36 nm in length), symmetrically surrounding a central triangular core (Fig.3). The rods are made up from stacks of disks (each 11 nm in diameter and 6 nm in thickness), which are hexameric subcomplexes and subdivided into two 3 nm thick trimeric subcomplexes, the fundamental building blocks of the rods. The core is constructed from two to five (usually three, both in hemidiscoidal and hemiellipsoidal phycobilisomes)

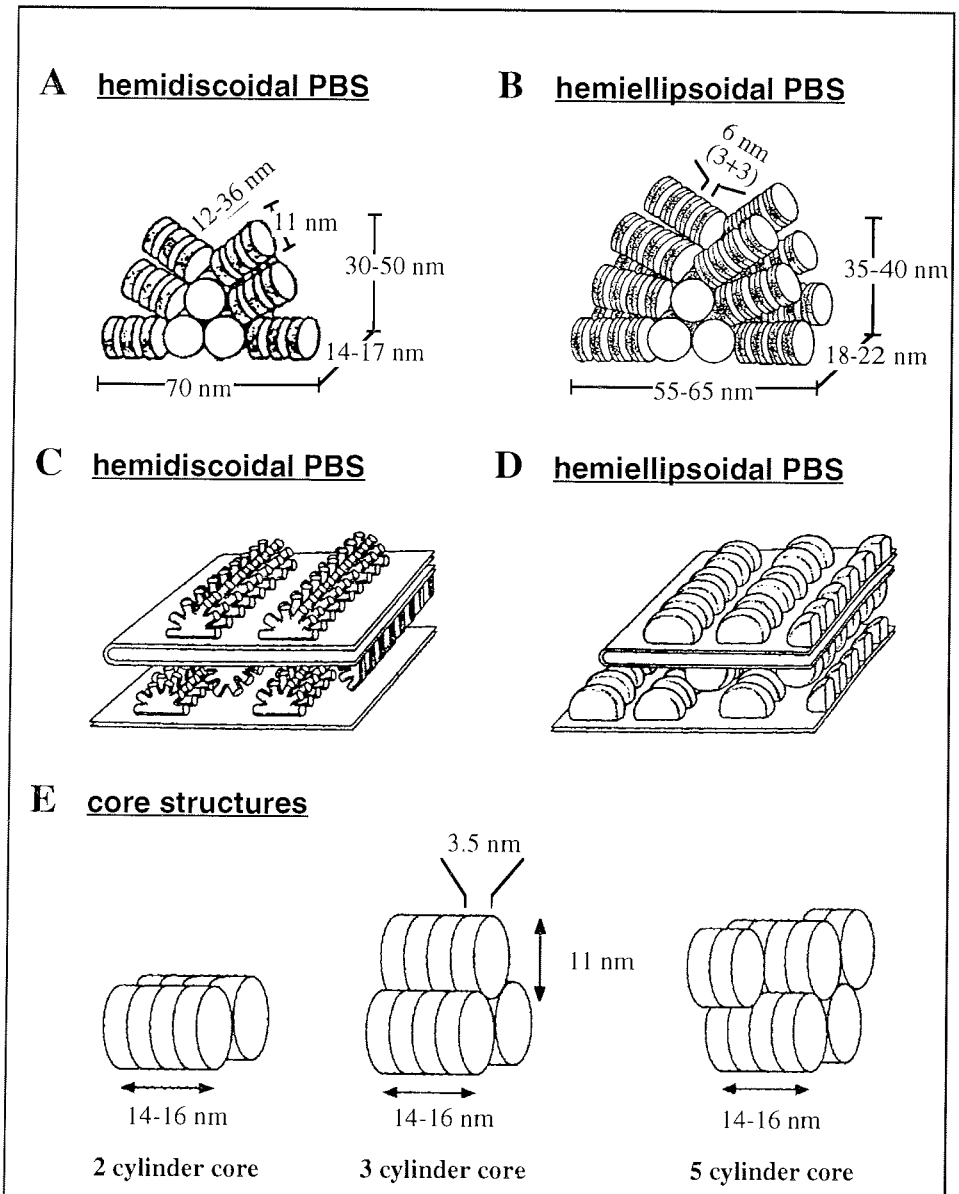


**Fig. 3.** Phycobilisome (PBS) structures. **A,** Hemidiscoidal PBS; **B,** hemiellipsiodal PBS; model of PBS arrangement and view on the thylakoid membrane with **C,** hemidiscoidal and **D,** hemiellipsiodal PBS; **E,** PBS core structures, most common is a tricylindrical core; the pentacylindrical core has two half cylinders. (From Wehrmeyer 1983, Mörschel 1991, Sidler 1994 and Ducret et al. 1998; modified)

cylindrical subcomplexes, each composed of usually four stacked discs (each 11 nm in diameter and about 3.5 nm in thickness). In the case of the pentacylindrical core three four- and two two-stacked dics were found (Ducret et al. 1996, 1998). The cylinders are oriented parallel to the thylakoid membrane. The hemidiscoidal phycobilisomes have a basal length of about 70 nm, a height of 30-50 nm, a thickness of 14-17 nm and a molecular mass of 4.5-15 MDa and contain 300-800 chromophores (Zuber 1987).

The more complex hemiellipsoidal phycobilisome type is mainly observed in advanced red algae (Lichtlé and Thomas 1976, Gantt and Lipschultz 1980), but also in the unicellular *Porphyridium cruentum* (Gantt 1981, Gantt and Lipschultz 1972, Redecker et al. 1993) and in few cyanobacteria (Wehrmeyer et al. 1988, Westermann et al. 1993). In face view, these phycobilisomes appear similar to the hemidiscoidal phycobilisomes, but contain almost the double amount of rods. Therefore, the phycobilisomes are at least twice as thick as the hemidiscoidal phycobilisomes, resulting in a more globular shape. The phycobilisomes have a basal length of 55-65 nm, a height of 35-40 nm, a thickness of about 18-22 nm and a molecular mass up to 30 MDa (Lange et al. 1990, Sidler 1994).

Phycobilisomes have been found to be regularly arranged in parallel rows on the thylakoids, and freeze-fracturing has shown that they are associated with intramembraneous particles (Mörschel and Mühlethaler 1983, Mörschel and Rhiel 1987). Each phycobilisome is attached to membrane complexes of 20 x 10 nm, which are divided by a central furrow in two 10 x 10 nm particels, are supposed to represent two reaction centres of photosystem II (RC II; Giddings et al. 1983, Manodori et al. 1984, 1985). However, in red algae and cyanobacteria, containing hemidiscoidal phycobilisomes, the PS II complexes are organised in dimers (1 dimer = about 20 x 10 x 10 nm = two RC II) that bind a single phycobilisome on the top (Giddings et al. 1983, Mörschel and Schatz 1987, Bald et al al. 1996), whereas hemiellipsoidal phycobilisomes of red algae are coupled to tetrameric PS II complexes (two dimers, four RC II) (Cunningham et al 1990; Kursar and Albert 1983, Lange et al. 1990). Usually, the light harvested by phycobilisomes is transferred directly to RC II, but under certain light conditions it may also be conducted directly to RC I (Bald et al. 1996, van Thor et al. 1998, MacColl 1998).

### 1.2.2. Phycobiliproteins

Phycobiliproteins were discovered almost 150 years ago by Cramer (1862). The chemical nature of phycoerythrin and of phycocyanin was described by Molisch (1894, 1895).

Phycobiliproteins show absorption bands in the visible light between 450-665 nm and commonly an ultraviolet absorption band at 330 nm. Thus, they fill the "green window" in the absorption spectrum of chlorophyll *a*. Phycobiliproteins are universally composed of α- and β-polypeptide subunits in equimolar (1:1) stoichiometry and with a molecular weight of 16-18 and 18-20 kDa, respectively, with one or more chromophores covalently attached (Stadnichuk 1995).

The chromophores of the phycobiliproteins are known as phycobilins (reviewed in Stadnichuk 1995, MacColl 1998). Four types of phycobilins are known and they are characterised by their absorption maxima: phycourobilin (PUB, 495 nm), phycoerythrobilin (PEB, 550 nm), phycoviolobilin (PVB, 590 nm) and phycocyanobilin (PCB, 661 nm). The chemical structure of these chromophores and their modes of linkage to the polypeptide chains are shown in Fig. 4. They are open-chain tetrapyrroles and belong to the same chemical family as chlorophyll and heme, but in contrast, they are metal-free. They are singly covalently bound to a cysteinyl residue of the polypeptide chain by a thioether linkage via the modified ethyl side group of pyrrole ring A, or doubly bound via pyrrole ring A and D. The spectroscopic properties of the phycobilins are primarily a function of their covalent structure and of their environment: the greater the number of conjugated double bounds, the greater the absorption in longwave light. There are five conjugated double bonds in the yellow-coloured PUB, six in the red-coloured PEB, seven in the purple-coloured PVB, and nine in the blue-coloured PCB. Their extinction coefficients are summarised in Table I.

The combination of these four phycobilins lead to a number of phycobiliprotein variants as summarised in Table II, but three major groups can be classified, according to their chromophore content, spectroscopic properties and immuno chemical response:

(1) Phycoerythrin (PE): Phycoerythrin appears orange-pink and absorbs light maximally between 560- 575 nm. It is the main phycobiliprotein in red algae. Three groups and ten types of phycoerythrins are known: RI-, RII-, RIII-phycoerythrin, BI- and BII-phycoerythrin, C- phycoerythrin, CUI-, CUII-, CUIII-, CUIV-phycoerythrin. The prefixes originally indicated the type of source organism: R-Rhodophytan; B- Bangiophycean; C- cyanobacteria and CU-cyanobacterial plus urobilin-like chromophore. However, they now describe only the spectral properties of phycobiliproteins. As shown in Table II, all PE types bind five or six chromophores of one or two chromophore types (PEB and/or PUB). In red algae, only R- and B-PE are found; the different spectral properties are illustrated in Fig. 5. At 495 nm, B-PE shows only a shoulder, whereas R-PE exhibits a clear extra peak. R-PE is wide-spread within the advanced Florideophyceae, whereas B-PE occurs

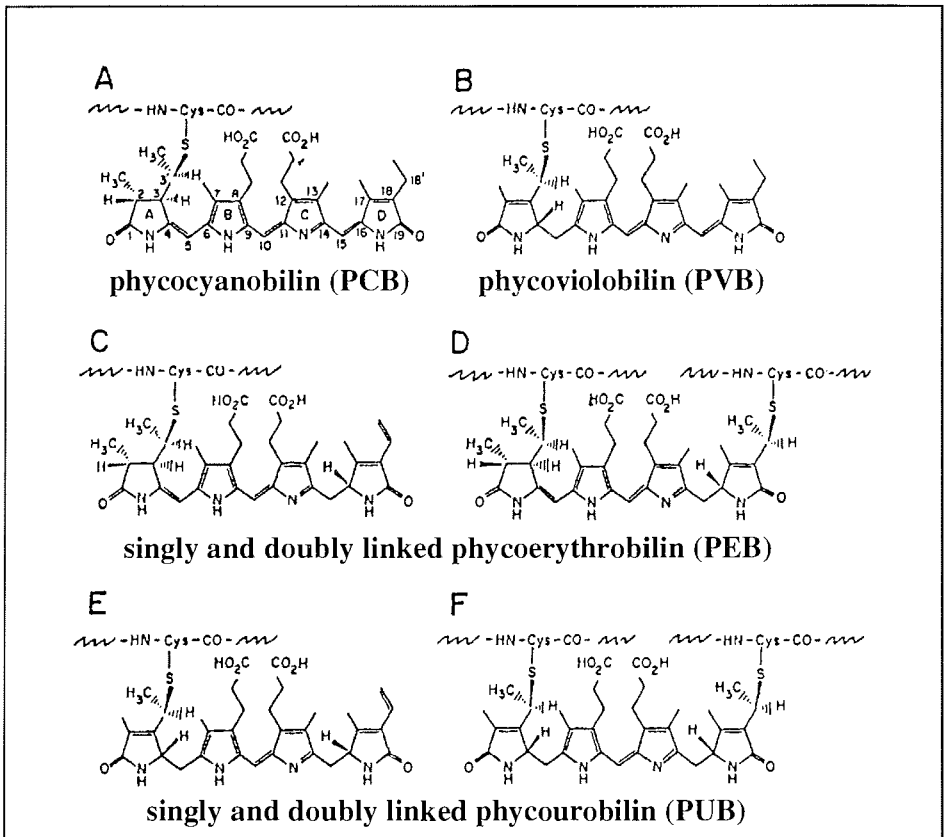


**Fig. 4.** Chemical structures of phycobilins, the chromophores of phycobiliproteins. They are singly or doubly linked by thioether-linkages to phycobiliproteins. **A**, Phycocyanobilin (PCB); **B**, phycoviolobilin (PVB); **C**, phycoerythrobilin (PEB); **D**, phycourobilin (PUB). (From Glazer 1988)

**TABLE I.** Millimolar extinction coefficients of polypeptide-bound phycobilins

| | | e ($mM^{-1}$ $cm^{-1}$) at wavelength | | | |
|---|---|---|---|---|---|
| Phycobilin | | 495 nm | 550 nm | 590 nm | 660 nm |
| Phycourobilin | PUB | **38.6** | 0 | 0 | 0 |
| Phycoerythrobilin | PEB | 18.3 | **53.7** | 8.5 | 0 |
| Phycoviolobilin* | PVB | 6.8 | 28.4 | **38.6** | 0 |
| Phycocyanobilin | PCB | 1.45 | 6.0 | 16.2 | **35.4** |

*new name for phycobiliviolin (PXB); main absorption is marked bold. Extinction coefficients for PUB, PEB and PVB determined in 10 mM aqueous trifluoroacetic acid and for PCB in 8 M aqueous urea, pH 1.9. Phycobilin absorption spectra in these two solvents are both qualitatively and quantitatively very similar. (From Glazer 1988, modified)

**TABLE II.** The occurence and location of phycobilins in the α-and β-subunits of phycobiliproteins. *Position in the amino acid sequence of the subunit.

| | occurence | | α-subunit | | | β-subunit | | |
|---|---|---|---|---|---|---|---|---|
| Phycobiliprotein | red algae | cyano-bacteria | α75* | α84 | α140 | β50/61 | β84 | β155 |
| APC | x | x | - | **PCB** | - | - | **PCB** | - |
| R-I-PC | x | x | - | PCB | - | - | **PCB** | PEB |
| R-II-PC | x | x | - | PEB | - | - | **PCB** | PEB |
| R-III-PC | x | x | - | PUB | - | - | **PCB** | PCB |
| C-PC | x | x | - | PCB | - | - | **PCB** | PCB |
| PEC | - | x | - | PVB | - | - | **PCB** | PCB |
| B-I-PE | x | x | - | PEB | PEB | PEB | **PEB** | PEB |
| B-II-PE* | x | x | | | | | | |
| R-I-PE | x | x | - | PEB | PEB | PUB | **PEB** | PEB |
| R-II-PE* | x | x | | | | | | |
| R-III-PE* | x | x | | | | | | |
| C-PE | - | x | - | PEB | PEB | PEB | **PEB** | PEB |
| CU-I-PE | - | x | - | PUB | PUB | PUB | **PEB** | PUB |
| CU-II-PE | - | x | - | PEB | PUB | PUB | **PEB** | PEB |
| CU-III-PE | - | x | PUB | PUB | PUB | PUB | **PEB** | PEB |
| CU-IV-PE | - | x | PUB | PEB | PEB | PUB | **PEB** | PEB |

APC, allophycocyanin; PC, phycocyanin; PE, phycoerythrin; PCB, phycocyanobilin; PEB, phycoerythrobilin; PUB, phycourobilin; PVB, phycoviolobilin. Permanent positions are marked bold: APC bear always PCB at position α84 and β84. All PC-types contain PCB at position β84. All PE-types bear PEB at β84. * attachment sites not known jet. (From Stadnichuk 1995, modified)

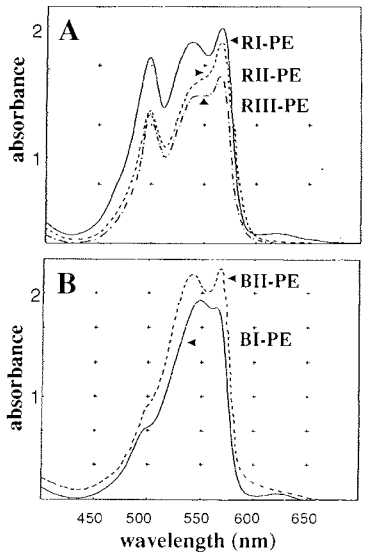


**Fig. 5.** Spectral types of phycoerythrin (PE) in red algae. **A,** R-PE types: RI-PE from *Ceramium rubrum*, RII-PE from *Palmaria palmata*, RIII-PE from *Phycodrys rubens*. **B,** B-PE types: BI-PE from *Audouinella proskaueri*, BII-PE from *Rhodosorus marinus*. (From Rennis and Ford 1992, modified)

mainly in the primitive Bangiophyceae (Glazer et al. 1982; Honsell et al. 1984, Rennis 1991, Rennis and Ford 1992). Recently, a novel R-PE, called R-PE IV, was discovered in the Antarctic red alga *Phyllophora antarctica* (MacColl et al. 1996, MacColl et al. 1999. Cyanobacteria contain mainly C-PE and CU-PE, but R-PE has also been found.

(2) Phycocyanin (PC): Phycocyanin appears lilac and absorbs light maximally between 615-640 nm. It is the main phycobiliprotein in cyanobacteria, but is also essential in red algae. Three groups and five types of phycocyanins are known: RI-, RII-, RIII-phycocyanin, C-phycocyanin and phycoerythrocyanin (PEC). All PC types bear three chromophores, most commonly PCB and PEB (but PUB in RIII-PC and PVB in PEC). In red algae, mainly R-PC but also C-PC were found. So far, RI-PC has only been characterised from red algae and not in cyanobacteria (Sidler 1994). RI-PC binds the chromophores PCB and PEB, accordingly two absorption maxima are found at 555 nm and 619 nm. In contrast, C-PC has only PCB and thus only one absorption maximum at 619 nm. Cyanobacteria contain small amounts of either PEC or PE.

(3) Allophycocyanin (APC): Phycocyanin appears turquoise and absorbs light maximally between 650-655 nm. APC assembles the phycobilisome core and three types have to be distinguished: allophycocyanin (APC), allophycocyanin-B (APC-B) and the so called $\beta^{18.3}$-subunit complex. APC exclusively contains two PCB chromophores. APC and small amounts of APC-B and $\beta^{18.3}$-subunit complex constitute the phycobilisome core in red algae (Redecker et al. 1993, Reuter and Wehrmeyer 1990, Reuter et al. 1990) and in cyanobacteria (Ducret et al. 1996). APC, APC-B and the $\beta^{16.5}$-complex have different fluorescence emission maxima at 660 nm, 670 nm and 680 nm, respectively. Consequently, APC-B was suspected to be a terminal energy emitter from the phycobilisomes to the Chl-proteins of PSII and/or PSI. However, APC-B is 'only' the second terminal emitter and plays a critical role in energy transfer from the phycobilisome to PS I and in the partitioning of light energy between the PS I and PS II reaction centres (Sidler 1994, Bald et al. 1996, Thor et al. 1998).

The polypeptide components of the phycobiliproteins are generally composed of α- and β-polypeptide chains/subunits in equimolar (1:1) stoichiometry, and with a molecular weight of 14-22 kDa. Nevertheless, recently a novel phycoerythrin lacking the α-subunit was found in *Rhodella reticulata* strain R6 (Thomas and Passaquet 1999). High resolution crystallographic studies of B-PE from *Porphyridium sordidum* and *P. cruentum* (Ficner et al. 1992, Ficner and Huber 1993), of C-PC from cyanobacteria

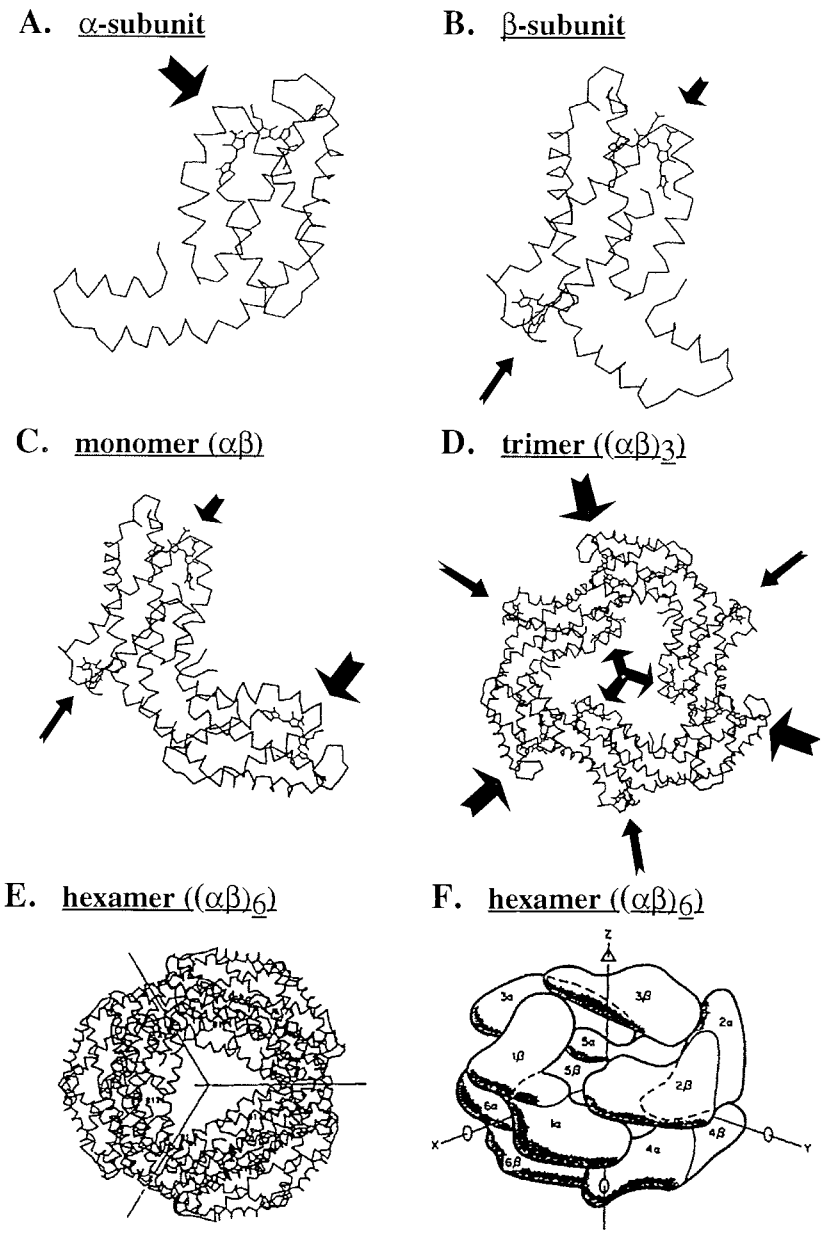


**Fig. 6.** Crystal structure of a phycobiliprotein (C-phycocyanin from cyanobacteria). **A,** α-subunit; **B**, β-subunit; **C**, monomer (αβ); **D**, trimer $((\alpha\beta)_3)$; **E**, hexamer $((\alpha\beta)_6)$, caused by face-to-face aggregation of two trimers; **F**, schematic side view of a $(\alpha\beta)_6$ hexamer, the subunits are labelled 1 to 6 with respect to the symmetry operation. Chromophore position are marked with arrows. (After Schirmer et al. 1985, 1986, 1987; from Sidler 1994, modified)

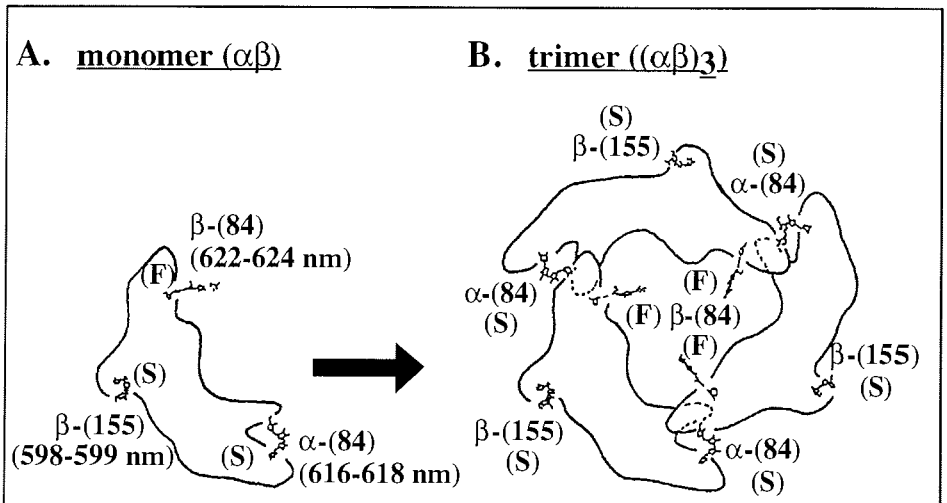


**Fig. 7.** A model of energy transfer in the C-phycocyanin trimer. **A,** monomer (αβ), the chromophores at position α-84 and β-155 are sensitizing (S) chromophores, whereas the chromophore at position β-84 is a fluorescing (F) chromophore. In the case of C-PC, the shortest wavelength absorbing chromophore was PCB at β-155 ($OD_{max}$ 598-599 nm), the middle-absorbing chromophore was PCB at α-84 ($OD_{max}$ 616-618 nm ) and the longest wavelength absorbing chromophore was PCB at β-84 ($OD_{max}$ 622-624 nm). **B,** trimer $((\alpha\beta)_3)$, the sensitizing peripheral PCB at β-155 (S) transfer excitation energy to the fluorescing PCB at β-84 (F) of the same monomer. The sensitizing PCB at α-84 transfer excitation energy to the fluorescing PCB at β-84 (F) of the neighbouring monomer. All fluorescing chromophores at β-84 (F) are suggested to transfer the excitation energy downhill to the APC core. (After Mimuro et al. 1986a, from Sidler 1994, modified)

(Schirmer et al. 1985, Schirmer et al. 1987, Duerring et al. 1991), of PEC from cyanobacteria (Duerring et al. 1990) and of APC from cyanobacteria (Brejc et al. 1995) are known. The well described crystal structure of C-PC from *Mastigocladus laminosus* (cyanobacterium) is shown in Fig. 6, and is quite similar to B-PE. The α- and β-subunits have very similar tertiary structures. One to three chromophores are covalently attached to the polypeptide moieties at special binding sites (amino acid position in α: 75, 84 and/or 140; β: 50/61, 82 and/or 155, Table II). In the case of C-PC, the α-subunit bears one chromophore (Fig. 6A) and the β-subunit bears two chromophores (Fig. 6B). The phycobiliprotein monomers (αβ) (Fig. 6C) look like a boomerang and the three chromophores are located at the periphery of the molecules. The chromophore amount of a phycobiliprotein is always related to this structure. In phycobilisomes, the phycobiliproteins are organised in trimers $((\alpha\beta)_3)$ and hexamers $((\alpha\beta)_6)$, the fundamental assembly units of the peripheral rod and core, substructures of phycobilisomes. The

trimers (Fig. 6D) form a ring shaped aggregate with a diameter of 11 nm, a thickness of 3-3.5 nm and with a central hole of 3 nm in diameter. Some chromophores project into the central cavity of the trimer, whereas others remain at the periphery. This chromophore arrangement makes an efficient energy transfer in the rods possible, as proposed by Mimuro et al. (1986a, 1986b) and demonstrated in Fig. 7. The hexamers (Fig. 6E and F) are formed by face-to-face aggregation of the trimeric disks and include a linker polypeptide in the central cavity (not shown). Accordingly, the above described electron microscopic discs in phycobilisomes are hexameric subunit complexes ($(\alpha\beta)_6$), composed of two trimeric subunit complexes ($(\alpha\beta)_3$).

In general, PE hexamers are located at the periphery of the rods, PC trimers at the inner part of the rods and APC trimers in the core. Therefore, an unidirectional radiationless excitation energy transfer from the short-wavelength (PE) to long-wavelength (APC) absorbing pigments in the phycobilisome is possible and occurs with an efficiency of more than 95 % (Mandori et al. 1984, Mandori et al. 1985, Glazer 1989).

### 1.2.3. Linker polypeptides

The linker polypeptides induce the face to face aggregation of trimers to hexamers, and cause the tail-to tail joining of hexamers to peripheral rods and core-cylinders. They induce the assembly of the phycobilisome core and its attachment to the thylakoid membrane and connect the rods to the core. The linker nomenclature is based on the molecular mass or the associated phycobiliprotein (e.g. $L_{CM}^{120}$ or $L_{CM}^{APC}$). The phycobilisome model in Fig. 8 demonstrates the nomenclature and function of the linker polypeptides.

According to their structural function in the phycobilisome, linker polypeptides are divided into four groups (Glazer 1985, Reuter and Müller 1993):

(1) rod linkers ($L_R$) are involved in the assembly of the peripheral rods and have molecular masses between 27-37 kDa and 8-14 kDa;

(2) rod-core linkers ($L_{RC}$) attach the rods to the core and have molecular masses between 27-37 kDa;

(3) core linkers ($L_C$) assemble APC and APC-B trimers in the core and have a molecular mass of 8-14 kDa;

(4) core core membrane linkers ($L_{CM}$) co-ordinate the assembly of APC trimers and have a molecular mass of 70-120 kDa. The molecular mass directly determines the core

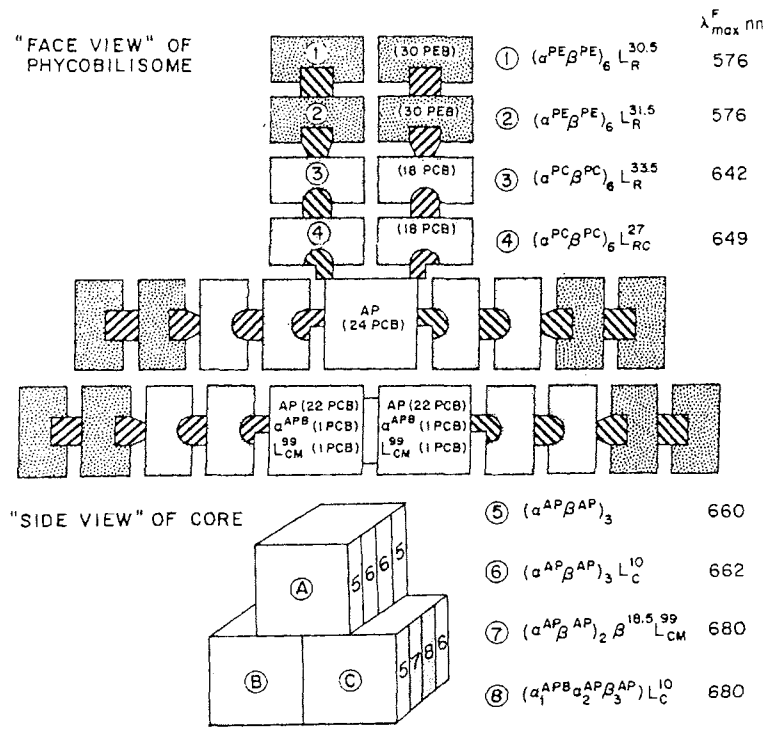


**Fig. 8.** Model of a hemidiscoidal phycobilisome from the cyanobacterium *Synechocystis* 6701. Each rod is made up of four hexameric phycobiliprotein complexes ($(\alpha\beta)_6$; PE, phycoerythrin; PC, phycocyanin). The core is made up of twelve trimeric complexes ($(\alpha\beta)_3$; AP, allophycocyanin; APB, allophycocyanin B). Each hexamer and trimer are attached through its specific linker polypeptide: $L_C$, core linker; $L_{CM}$, core membrane linker; $L_R$, rod linker; $L_{RC}$, rod core linker (the supercripts denote their apparent molecular masses in kDa). In the hexameric and trimeric boxes, the number of chromophores (PEB, phycoerythrobilin; PCB, phycocyanobilin) are shown. The fluorescence emission maximum ($\lambda^F_{max}$ in nm) is given for each of the eight subcomplexes isolated from this phycobilisome. (From Glazer and Clark 1986, modified)

structure/size. The number of core cylinders formed by linking of APC trimers is illustrated in Fig. 3E. A $L_{CM}^{72}$ forms a two cylinder core, a $L_{CM}^{94}$ and $L_{CM}^{99}$ a three cylinder core, and a $L_{CM}^{128}$ results in a five cylinder core (Sidler 1994). Two copies of $L_{CM}$ are present per phycobilisome core. Furthermore, the $L_{CM}$ attaches the phycobilisome to the thylakoid membrane and transfers excitation energy from the phycobilisome to Chl *a* associated with the reaction centre of PS II (Redlinger and Gantt 1982, Mimuro et al. 1986a, Glazer 1989, Gindt et al. 1992, Zhao et al. 1992). $L_{CM}$ is the main terminal emitter in the phycobilisome core and transfers about 75 %

of the excitation energy, while APC-B is the second terminal emitter and distributes the remaining excitation energy between PSI and PS II (in PS II probably involving $L_{CM}$) (Zhao et al. 1992, Bald et al. 1996, Thor et al. 1998).

Besides their structural function, all linker polypeptides are thought to be involved in energy transfer. They modulate the spectral properties of the phycobiliprotein trimers and hexamers by interacting with the chromophores, or indirectly by changing the chromophore environment, thereby causing a minor red-shift in the absorption and fluorescence maxima of the phycobiliprotein-linker polypeptides-complexes (Glazer 1985, Mimuro et al. 1986b, Watson et al. 1986, Glazer and Clark 1988). These minimal changes support the unidirectional transfer of excitation energy in the phycobilisome from the periphery of the rods to the core.

The linker polypeptides are basic (ca. pI 6.0 - 8.5), whereas the phycobiliproteins are more acidic (ca. pI 6.3 - 4.3) (Gantt 1990, Aráoz et al. 1998). The linkers are expected to be more hydrophobic and positively charged at physiological pH, whereas the phycobiliproteins are extremely hydrophilic and negatively charged. Therefore linker polypeptides and phycobiliproteins interact by hydrophobic and charge-charge interactions, which would also explain the hydrophilic nature of phycobilisomes (Sidler 1994, Zilinskas and Glick 1981).

Generally, linker polypeptides are uncoloured. With exception of the large core-membrane linker ($L_{CM}$), which carry a single PCB chromophore, and the so called γ-subunits. γ-subunits were originally found in PE of red algae (Glazer and Hixon 1977), however, recent studies have demonstrated their occurrence in R-PE of some marine cyanobacteria (Wilbanks and Glazer 1993, Swanson et al. 1991). As linker polypeptides, they are located in the cavity of the hexameric PE structure. The γ-subunits of red algae generally carry four or five chromophores (PUB and/or PEB), whereas in cyanobacteria only one (PUB) was found (Klotz and Glazer 1985, Ong and Glazer 1991, Stadnichuk et al. 1993, Wilbanks and Glazer 1993, Apt et al. 1993). In red algae, up to three different γ-subunits have been identified. Their pI of 8.9 is typically high as of other linker polypeptides (Sidler 1994). So, the γ- subunits are bifunctional, they act as light-harvesting phycobiliproteins and as rod linker polypeptides.

### 1.2.4. Genes encoding the phycobilisome components

In red algae, most genes encoding the phycobilisome components (including α- and β-subunit) are localised in the chloroplast genome (Shivji 1991, Bernard et al. 1992,

Valentin et al. 1992, Reith and Munholland 1993, Apt and Grossman 1993a, 1993b, 1993c, Roell and Morse 1993, Kim and Fujita, 1997). In contrast, genes encoding the PE linkers (including the γ-subunits) are localised on the nuclear DNA, and are translated on 80S ribosomes in the cytoplasm (Egelhoff and Grossman 1983, Apt et al. 1993). The γ-subunit is suggested to be transported into the plastid by a mechanism similar to that of higher plants (Apt et al. 1993). Genes encoding the phycobilisome components in cyanobacteria are summarised and reviewed in Houmard and Tandeau de Marsac (1988), Bryant (1991) and Sidler (1994).

## 1.3. Thesis outline

*P. decipiens* is endemic and one of the most common Antarctic red algae. In the last ten years many studies on growth, photosynthesis and storage compounds were performed to understand the life strategy, that makes this alga so successful in its extreme habitat. This thesis focuses on the acclimation processes of the photosynthetic apparatus to the seasonally strongly varying light conditions from the physiological down to the molecular biological level. In five original research publications, the phycobilisomes from *P. decipiens* were isolated and characterised, and their dynamics in response to the main changing Antarctic environmental factors, the seasonal changing daylengths and the extended winter sea-ice covering, was investigated and related to the phycobiliprotein contents of the cell as well as the optimal quantum yield and the photosynthetic performance.

Publication 1 describes the unexpected finding of two hemiellipsoidal phycobilisome forms in *P. decipiens*, isolated on discontinuous sucrose gradients. Both phycobilisome forms were characterised by absorption and fluorescence spectroscopy. The trimeric and hexameric subunit complexes were separated by native PAGE and were also characterised by absorption and fluorescence spectroscopy. The α-,β- and γ-subunits and the linker polypeptides were identified by SDS-PAGE, and their apparent molecular masses were calculated by densitometric analysis. The phycobilisome structure was shown in electron micrographs of negatively stained phycobilisomes. The techniques used had to be developed and modified to the special demands of phycobilisome isolations from Antarctic seaweeds.

Publications 2 and 3 focus on the influence of seasonally fluctuating Antarctic daylengths on the optimal quantum yield, the photosynthetic performance, the Chl *a* and

phycobiliprotein content of the cell and the assembly of phycobilisomes, studied in a year-round culture experiment in monthly intervals.

Publications 4 and 5 report the influence of winter sea-ice cover, simulated by an extended dark period (from 2nd April to 1st October), and of subsequent re-exposure to light under seasonally fluctuating Antarctic daylengths on the same parameters, the optimal quantum yield, the photosynthetic performance, the Chl *a* and phycobiliprotein content of the cell and the phycobilisome assembly, studied in a parallel year-round culture experiment in monthly intervals. In October, the acclimation to light was investigated in more detail and subsamples were taken in short time intervals during the first 4 weeks after re-illumination of dark-exposed plants.

This is the first study, in which phycobilisomes from an Antarctic macroalga were isolated and characterised. It is the first time, that the dynamic of phycobilisome assembly in response to seasonally changing daylength was demonstrated. At last, it is even the first time, that the degradation of phycobilisomes was monitored under prolonged darkness. It is one of the few studies, in which phycobilisome assembly is related to photosynthetic performance. Together with the physiological studies it gives detailed insight into the life strategy of this ecologically important endemic Antarctic macroalga.

# 2. MATERIAL AND METHODS

## 2.1. Culture conditions and experimental design

Tetraspores of *Palmaria decipiens* (Reinsch) Ricker were collected 1987 on King George Island (South Shetland Islands, Antarctica, Fig. 9). The developing male gametophytes were then cultivated in the laboratory under seasonally fluctuating daylengths (mimicking field conditions, Pub. 3, Fig. 1) at the Alfred Wegener Institute in Bremerhaven, Germany (Wiencke 1988, Wiencke 1990a). *Palmaria palmata* (Linnaeus) Kuntze was collected in Britanny and cultivated under a constant daylength of 16 h light and 25 µmol photons $m^{-2}$ $s^{-1}$ at 10 °C. *Porphyridium cruentum* Naegeli and *Rhodella violacea* Kornmann (obtained from J. Marquardt, Philipps University of Marburg) were grown at 20 °C in an artificial sea water medium (18 ‰, pH 7.3, Jones et al. 1963) under 100 µmol photons $m^{-2}$ $s^{-1}$ and 16 h light per day.

1. Long-term experiment - The influence of seasonally fluctuating daylengths: Numerous male gametophytes of *P. decipiens* (Fig. 10) were grown in 24 3 l glass beakers for about two years under simulated Antarctic daylength conditions (Publ. 3, Fig. 1) and under a constant photon fluence rate of 25 µmol photons $m^{-2}$ $s^{-1}$ using daylight fluorescent tubes (Osram L58/W19). Temperature was maintained constant at 0±1 °C. The cultures were grown in Provasoli enriched North Sea water (Provasoli 1966, 34 ‰, pH 8), aered with pressed air. To ensure sufficient nutrient supply, the culture medium was changed every second week. After about one year, we started under Antarctic March conditions (end of Antarctic sommer) to harvest individual plants from different glass beakers in monthly intervals for photosynthesis and pigment measurements as well as for phycobilisome isolations.

2. Long-term experiment - The influence of winter sea-ice cover: To simulate winter sea-ice cover, 12 glass beakers with plants from the 1. experiment were placed in complete darkness for six months (from 2nd April to 1st October). Afterwards, they were re-illuminated according to the light conditions of the 1. experiment (Publ. 5, Fig. 1). Every month, individual plants were harvested from different glass beakers and used for photosynthesis and pigment measurements as well as for phycobilisome isolations. Under Antarctic October conditions the acclimation to light was investigated in more detail and samples were taken after 1, 3, 5, 7, 10, 14, 17, 21, 24 and 28 days of re-illumination of dark-incubated plants.

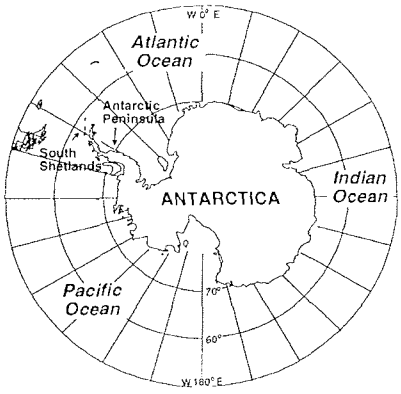


**Fig. 9.** Map of Antarctica. King George Island (62°12'S, 58°58'W) belongs to the South Shetland Islands adjacent to the Antarctic Peninsula

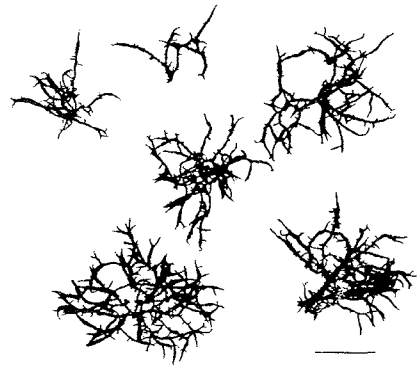

**Fig. 10.** Habit of *Palmaria decipiens*, male gametophytes. Scale bar = 2 cm.

## 2.2. Measurements of optimal quantum yield and photosynthetic performance

Optimal quantum yield and photosynthetic performance were determined by using chlorophyll a fluorescence measurements conducted pulse-amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) connected to a personal computer. The system is based on the principle described by Schreiber et al. (1986 and 1994).

The optimal quantum yield of photosystem II was determined as the ratio of variable to maximal fluorescence ($F_v/F_m$) of temporarily dark incubated plants according to Bischof et al. (1999) and Hanelt (1998). Thallus pieces were fixed with a net close to the fibre optic probe of the fluorometer and inserted into a custom-made metal cuvette filled with seawater at 0 °C. First, a 5-s far red pulse, which selectively excites photosystem I (≈ 30 µmol photons $m^{-2}$ $s^{-1}$, 735 nm) was applied to oxidise the electron transport chain, and then the algae were further kept for 5 min in the cuvette in darkness. Subsequently, a pulsed dim red light (≈ 0.3 µmol photons $m^{-2}$ $s^{-1}$, 650 nm) was applied to measure the minimal fluorescence ($F_o$; all reaction centres of photosystem II are oxidised or "open"), followed by a short pulse of saturating white light (0.6 s, ≈8000 µmol photons $m^{-2}$ $s^{-1}$) to measure the maximal fluorescence ($F_m$; all reaction centres of photosystem II are reduced or are "closed") of this temporarily dark-incubated sample. The variable fluorescence ($F_v$) was determined by use of the following equation: $F_v = F_m - F_o$. Thus, the optimal quantum yield is given by: $F_v/F_m = (F_m - F_o)/F_m$ .

Subsequently, a light response curve of fluorescence was recorded with increasing actinic red light irradiances of 3.5 to 350 µmol photons $m^{-2}$ $s^{-1}$ (650 nm) and used to calculate the maximal electron transport rate ($ETR_{max}$). At each irradiance the effective quantum yield of photosystem II ($\Delta F/F_m'$) was determined. Therefore, the plants were temporarily irradiated with the lowest actinic red light irradiance (650 nm, 3.5 µmol photons $m^{-2}$ $s^{-1}$). After stabilisation of the fluorescence level, the steady state fluorescence ($F_s$) was measured and a pulse of saturating white light was applied to measure the maximal fluorescence of this light-incubated plant ($F_m'$), followed by 5 s of darkness to measure the minimal fluorescence of this light-incubated plant ($F_o'$). Every 30 s, the actinic irradiance was further increased and the respective $F_s$, $F_m'$ and $F_o'$ were determined. For each irradiance, the effective quantum yield of photosystem II ($\Delta F/F_m'$) was calculated using following equations: $\Delta F/F_m' = (F_m' - F_s) / F_m'$ (Genty et al. 1989). For each irradiance the relative electron transport rate (ETR) was calculated by multiplying the effective quantum yield with the respective photon flux density of actinic irradiance (PFD): $ETR = \Delta F/F_m' * PFD$ (Schreiber et al. 1994). Then, ETR were plotted against PFD (light response curve of ETR). The maximal electron transport rate ($ETR_{max}$) was calculated from the saturated part of these curve (plateau) by linear regression analysis. PFD on front of the fibre optics was measured using a cosine-corrected flat-head sensor (Quantum, Li 190 SA, Li-Cor, Lincoln, USA).

All measurements were conducted in sea-water at 0 °C using 8-10 different individual thalli of *P. decipiens* from different beakers at the beginning of daytime. In macroalgae, the results of fluorescence measurements are consistent with those of oxygen measurements (Hanelt 1992, Hanelt et al. 1992, Hanelt et al. 1994). $ETR_{max}$ can be used as an indicator for maximal photosynthetic performance.

## 2.3. Determination of phycobiliprotein and chlorophyll *a* contents

Phycobiliprotein contents of cells and of isolated phycobilisomes were determined after Rosenberg (1981) in six different individuals. Plant material was disrupted by grinding and then extracted in 0.1 M potassium phosphate buffer, pH 6.8. The extract was frozen and thawed several times to enhance the extraction process. The resulting extract was centrifuged and the absorbance of the supernant at 565 nm, 615 nm and 650 nm was determined and calculated using the following equations: $PE (\mu g/ml) = 123.5 * OD_{565} - 73.5 * OD_{615} - 16.3 * OD_{650}$, $PC (\mu g/ml) = 163.2 * OD_{615} - 117.1 * OD_{650}$ and $APC (\mu g/ml) = 165.6 * OD_{650} - 16.4 * OD_{615}$.

Chlorophyll *a* (Chl *a*) was extracted in N,-N dimethylformamide (DMF) according to Inskeep and Bloom (1985) and carried out with 6-8 different individuals.

## 2.4. Isolation and characterisation of phycobilisomes

***Isolation of phycobilisomes***

The suitability of the isolation protocols used for *Palmaria decipiens* was tested on the temperate *Palmaria palmata* and on the unicellular microalgae *Porphyridium cruentum* and *Rhodella violacea.* The phycobilisomes were isolated according to Nies and Wehrmeyer (1980) and Reuter and Wehrmeyer (1990). After disruption of *P. decipiens* and *P. palmata* by grinding and of *Porphyridium* and *Rhodella* by passing through a French pressure cell, the homogenates were incubated in 1 % (w/v) N,N-dimethyl-dodecylamine-N-oxide (LDAO, Fluka, Buchs, Switzerland), deoxyribonuclease I (DNase I, 80 mg $ml^{-1}$ extract) and 15 % (w/v) sucrose in 1.5 M potassium phosphate buffer, pH 6.8, for 30 min in the dark at room temperature with gentle stirring. LDAO highly efficient detaches the phycobilisomes from the thylakoid membranes, an effect of the excellent solubility of LDAO in high molar phosphate buffers (Reuter and Wehrmeyer 1990). To prevent protcolytic degradation, all buffers contained 1 mM Pefabloc SC (Boehringer, Mannheim, Germany) and 2 mM EDTA. Cell debris was removed by centrifugation for 30 min at 48000 g (Sorvall RC-5B, SS34, Du Pont de Nemours, Bad Homburg, Germany). The supernatant was layered onto a step sucrose gradient of 15, 25, 35 and 45 % (w/v) sucrose in 1.5 M potassium phosphate buffer, pH 6.8, and was ultracentrifugated at 280000 g and 13 °C for 22 h (L5-65, SW 40 Ti, Beckman, Fullerton, CA, USA). After centrifugation the phycobilisomes were collected with a syringe. An aliquot was directly used for fluorescence emission spectroscopy and for electron microscopy. The remaining phycobilisomes were pelleted by ultracentrifugation at 280000 g for 3 h (L5-65, Ti 50, Beckman), resuspended in 15 % (w/v) sucrose with 1 mM Pefabloc SC, frozen at -30 °C in aliquots, and used for subsequent electrophoresis.

Phycobilisomes have to be isolated and stabilised in high molarity buffers, like 0.75 M or 0.9 M potassium phosphate buffer, pH 6.8, with sucrose added (Gantt and Lipschultz 1972, Nies and Wehrmeyer 1980, Katoh 1988). Isolations of phycobilisomes from *P. decipiens* were tested in 0.75 M, 1.0 M and 1.5 M potassium phosphate buffer, pH 6.8 containing 15% (w/v) sucrose, and the best results in banding were obtained at the highest potassium phosphate concentration. The phycobilisomes from *P. decipiens* seem to be very fragile and need extremely high buffer concentrations for stabilisation

during the isolation process. The reason might be a high *in vivo* content of cryo-protective substances in *P. decipiens*.

As phycobilisomes are generally more stable at room temperature than at 4 °C, isolation is generally performed at room temperature. Isolation temperatures of 4-10 °C result in an uncoupling of phycobilisomes or a loss of allophycocyanin (Gantt et al. 1979, Mörschel and Rhiel 1987, Glazer 1988b). Even the phycobilisomes of the cold water species *P. decipiens* dissociated at 4 °C, and no intact phycobilisomes were detected in the gradients.

***Fluorescence and absorption measurements***

The intactness of isolated phycobilisomes was proven by an effective energy transfer to the terminal energy acceptor of the phycobilisomes in fluorescence emission spectra. Intact phycobilisomes emit with a maximum at 670-675 nm at room temperature and at 678-685 nm at -196 °C. In contrast, dissociated phycobilisomes show an increased fluorescence emission from phycoerythrin (575 nm), phycocyanin (640-650 nm) and allophycocyanin (660 nm) as well as a loss of the 670-680 nm peak (MacColl and Guard-Friar 1987). Fluorescence emission spectra were measured at 77 K and at room temperature in a spectrofluorometer (PTI, Photon Technology International, Lawrenceville, NJ, USA) with an excitation of 450 nm. Absorption spectra were recorded with a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan).

***Protein determination***

The protein content of isolated phycobilisomes was determined with a Bio-Rad-Protein Assay (Bio-Rad, Munich, Germany), according to the instructions of the manufacturer.

***Native polyacrylamide gel electrophoresis (native PAGE)***

The isolated phycobilisomes were separated into their subunit complexes by native PAGE on 2 mm thick gels containing gradients of 6-14 % (w/v) polyacrylamide (37.5:1 w/w acrylamide to methylenebisarcrylamide) and 7-14 % (w/v) sucrose, according to Reuter and Nickel-Reuter (1993). Sucrose stabilises the subcomplexes (Zilinskas and Glick 1981). The gel and electrode buffer was tris-boric acid (100 mM/ 73 mM, pH 8.6). The gel buffer contained 2 mM EDTA to inhibit the specific proteolytic enzymes, which are involved in the degradation of the linker polypepides (Reuter and Müller 1993).The electrophoresis was performed in a Mini Protean II cell (Bio-Rad, Munich, Germany) for 16 h on ice at a constant voltage of 200 V. The coloured bands were cut out and pigment proteins were electroeluted at 200 V in the same electrode buffer containing 7 % (w/v)

sucrose using a Centrilutor-Micro-Electroeluter (3000 MWCO, Amicon, Witten, Germany). Protein extracts were used for SDS-PAGE. Some gel strips were used for absorption and fluorescence spectral analysis.

***Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)***

The polypeptide composition of the isolated phycobilisomes and the subunit complexes were analysed on 15 % (w/v) polyacrylamide gels according to Reuter and Wehrmeyer (1988). For a better resolution of the linker polypeptides 6 % (v/v) isopropanol was added to the separation gel as described by Reuter and Nickel-Reuter (1993). Electrophoresis was performed on ice in a Mini Protean II cell (Bio-Rad) for 2 h at 200 V. Samples were solubilised at 80 °C for 10 min in a buffer containing 5 % (w/v) SDS, 2 % (w/v) dithiothreitol, 20 % (w/v) sucrose, 0.25 M Tris HCl, pH 6.8 and 0.02 % (w/v) Coomassie Brillant Blue G250. After electrophoresis, gels were fixed for 1 h in 7 % (v/v) acetic acid with 40 % methanol, stained overnight in 0.025 % (w/v) Coomassie Brillant Blue R250 in 7 % (v/v) acetic acid and destained in 7 % (v/v) acetic acid. For quantification of relative protein content the stained gels were scanned at 520-570 nm with a GS-700 Imaging Densitometer (Bio-Rad) with Multi-Analyst Software (Bio-Rad).

***Electron microscopy***

For fixation, the isolated phycobilisomes were immediately diluted 1:1 with isolation buffer containing 0.2 % (v/v) glutaraldehyde and 15 % (v/v) sucrose and stored for 2 h according to Wehrmeyer et al. (1993). They were then negatively stained with uranyl acetate and subsequently examined in a transmission electron microscope (EM 902, Zeiss, Oberkochen, Germany). The negative staining procedure was performed according to Nermut (1973) with the drop technique. 400 mesh formvar-coated grids were floated on a sample drop for 5 min, washed three times in distilled water and then floated again on a drop of 2 % uranylacetate for 1 min. The excess solution was removed with filter paper and the grids were subsequently viewed in the microscope.

# 3. PUBLICATIONS

## 3.1. List of publications

**Publication 1**: **Lüder UH**, Knoetzel J and Wiencke C. Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae). Physiologia Plantarum (2000) 112: 572-581.

**Publication 2**: **Lüder UH**, Knoetzel J and Wiencke C. Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. Polar Biology (2001) 24: 598-603

**Publication 3**: **Lüder UH**, Knoetzel J and Wiencke C. New insights into phycobilisome structure and their variability according to seasonally changing light conditions in the Antarctic red macroalga *Palmaria decipiens*. (prepared to be submitted).

**Publication 4**: **Lüder UH**, Wiencke C and Knoetzel J. Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta) - a study to simulate Antarctic winter sea ice cover. Journal of Phycology (2002) 38: 904-913.

**Publication 5**: **Lüder UH**, Knoetzel J and Wiencke C. Dynamics of phycobilisome assembly during and after six months of darkness in *Palmaria decipiens* (Florideophyceae) - a study to simulate Antarctic winter sea ice cover. (in preparation to be submitted).

## 3.2. Declaration about my working part of the publications

**Publications 1, 2, 3, 4 and 5** are all based on own laboratory experiments, planned, performed and analysed by myself. Each manuscript was written by myself. The co-authors J. Knoetzel and C. Wiencke have supervised my work and helped to review the manuscripts.

Methods for the isolation and characterisation of phycobilisomes described in **publications 1, 3 and 5** were established and adapted for the use of marine macroalgae by myself.

## *Publication 1*

Lüder UH, Knoetzel J and Wiencke C:

# Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae).

Physiologia Plantarum (2000) 112: 572-581.

PHYSIOLOGIA PLANTARUM 112: 572–581. 2001
Printed in Ireland—all rights reserved


ISSN 0031-9317

# Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae)

Ulrike H. Lüder[a,*], Jürgen Knoetzel[b,1] and Christian Wiencke[a]

[a]*Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany*
[b]*Institute of Cell Biology, Biochemistry and Biotechnology, University of Bremen, P.O. Box 33 04 40, D-28334 Bremen, Germany*
[1]*Present address: Department of Plant Biology, Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark*
**Corresponding author, e-mail: ulueder@awi-bremerhaven.de*

Received 12 September 2000; revised 28 February 2001

**The phycobilisomes (PBS), the light-harvesting antennae, from the endemic Antarctic red macroalga *Palmaria decipiens* were isolated on discontinuous sucrose gradients in two discrete bands and not in one as expected. To exclude methodical faults, we also isolated PBS from the temperate *Palmaria palmata* and the unicellular red algae *Porphyridium cruentum* and *Rhodella violacea*. In *P. palmata* the PBS were separated in two discrete bands, whereas the PBS from *Porphyridium* and *Rhodella* were found in one band. The double-banded PBS ($PBS_{up}$ and $PBS_{low}$) from *P. decipiens* were further characterized by absorption and fluorescence spectroscopy, native and SDS-PAGE as well as by negative staining. The phycobiliproteins RIII-phycoerythrin, RI-phycocyanin and allophycocyanin were identified and 3 γ-subunits were described. The $PBS_{up}$ and $PBS_{low}$ showed no significant differences in their absorption spectra and phycobiliprotein ratios although the negative stained $PBS_{low}$ were smaller. Differences were found in their low molecular mass subunit complexes, which are assumed to be r-phycoerythrin. The polypeptide pattern of the $PBS_{up}$ and $PBS_{low}$ showed no differences in the molecular masses of their subunits and linker polypeptides, but in their percentage distribution. The results suggest that the $PBS_{low}$ is a closer packed and $PBS_{up}$ a little more loosely aggregated hemiellipsiodal PBS form. We discuss the ecophysiological function of two PBS forms in *P. decipiens* and suggest advantages in the rapid acclimation to changes in environmental light conditions.**

## Introduction

Phycobilisomes (PBS) are supramolecular structures of pigment–protein complexes that function in cyanobacteria and in red algae as light-harvesting antennae. Their structure, composition, spectral properties and the energy flow were recently reviewed by various authors (Gantt 1990, Holzwarth 1991, Mörschel 1991, Reuter and Müller 1993, Sidler 1994, Talarico 1996, MacColl 1998, van Thor et al. 1998). Most studies were performed on cyanobacteria and unicellular red algal genera like *Porphyridium* or *Rhodella*. There have only been a few studies on macroalgae, mainly on genera belonging to the primitive red algal class Bangiophyceae (e.g. *Porphyra*). Comparatively very few studies were performed on advanced red macroalgae (Florideophyceae). The recent discovery of a novel phycoerythrin (PE) type, called R-PE IV, in the Antarctic red alga *Phyllophora antarctica* (MacColl et al. 1996, 1999) shows the need to investigate in more detail the light-harvesting antennae of macroalgae living under extreme conditions.

In the Antarctic, sublittoral algae are exposed to almost constant low water temperatures (−1.8 to +2.0°C) and high nutrient concentrations over the entire year. Therefore, the seasonal development of Antarctic macroalgae depends mainly on the seasonal variation of light conditions (Wiencke 1996).

*Palmaria decipiens* (Palmariales, Florideophyceae) is endemic and one of the most common Antarctic red macroalgae. Seasonal changes in the amount and in the ratios of phycobiliproteins in *P. decipiens* (Lüder et al. 2001) suggest changes in the PBS number and size and reflect a high potential of acclimation processes to environmental changes in light conditions. For this reason, we were interested to investigate the PBS in more detail. This is the first study in

*Abbreviations* – APC, allophycocyanin; PBS, phycobilisome; $PBS_{up}$ and $PBS_{low}$, upper and lower isolated PBS; PC, phycocyanin; PE, phycoerythrin; PEB, phycoerythrobilin; PUB, phycourobilin.

which PBS from an Antarctic macroalga were isolated and characterized by absorption and fluorescence spectral analysis, by native and denatured PAGE as well by negative staining. We report here the unexpected findings of two hemiellipsoidal PBS forms in *P. decipiens*. Two types of PBS were recently described only in *Porphyra umbilicalis* from the field (Algarra et al. 1990) and in *Porphyra yezoensis* during its ontogenesis (Shi et al. 1995). We discuss the ecophysiological role of two PBS forms in *P. decipiens*.

## Material and methods

### Algal material and culture conditions

*Palmaria decipiens* (Reinsch) Ricker was isolated on King George Island (South Shetland Islands, Antarctica) and cultivated as described by Lüder et al. (2001) under simulated Antarctic day length conditions (20 h light in summer and 5 h light in winter) under a constant photon fluence rate of 25 μmol photons $m^{-2}$ $s^{-1}$ using cool-white fluorescent tubes (L58/W19, Osram, Munich, Germany) and at a constant temperature of 0°C in Provasoli enriched North Sea water (34‰, pH 8). During sampling times, the algae were exposed to 16 h light per day (Antarctic November light conditions). *Palmaria palmata* (Linnaeus) Kuntze was isolated in Brittany and cultivated under a constant day length of 16 h and 25 μmol photons $m^{-2}$ $s^{-1}$ (L58/W19, Osram) at 10°C.

*Porphyridium cruentum* (Naegeli) and *Rhodella violacea* (Kornmann) were obtained from Dr Jürgen Marquardt from the Philipps University of Marburg and grown at 20°C in an artificial sea water medium (18‰, pH 7.3; Jones et al. 1963) at 100 μmol photons $m^{-2}$ $s^{-1}$ (L58/W19, Osram) and 16 h light per day.

### Isolation of PBS

The PBS were isolated according to Nies and Wehrmeyer (1980) and Reuter and Wehrmeyer (1990). Cells were disrupted by grinding (*Palmaria*) or by passing through a French pressure cell (*P. cruentum* and *R. violacea*). The homogenates were incubated in 1% (w/v) N,N-dimethyldodecylamine-N-oxide (LDAO; Fluka, Buchs, Switzerland), deoxyribonuclease I (DNase I, 80 mg $ml^{-1}$ extract) and 15% (w/v) sucrose in 1.5 *M* potassium phosphate buffer, pH 6.8, for 30 min in the dark at room temperature with gentle stirring. To prevent proteolytic degradation, all buffers contained 1 m*M* Pefabloc SC (Boehringer, Mannheim, Germany) and 2 m*M* EDTA. Cell debris was removed by centrifugation for 30 min at 48000 *g* (Sorvall RC-5B, SS34; Du Pont de Nemours, Bad Homburg, Germany). The supernatant was layered onto a step sucrose gradient of 15, 25, 35 and 45% (w/v) sucrose in 1.5 *M* potassium phosphate buffer, pH 6.8, and was ultracentrifugated at 280000 *g* and 13°C for 22 h (L5-65, SW 40 Ti; Beckman, Fullerton, CA, USA). After centrifugation the PBS were collected with a syringe. An aliquot was directly used for fluorescence emission spectroscopy and for electron microscopy. The remaining PBS were pelleted by ultracentrifugation at 280000 *g* for 3 h (L5-65, Ti 50; Beckman), resuspended in 15% (w/v) sucrose with 1 m*M* Pefabloc SC, frozen at −30°C in aliquots, and used for subsequent electrophoresis.

### Native PAGE

The PBS were separated into their subunit complexes by native PAGE on 2-mm-thick gels containing gradients of 6–14% (w/v) polyacrylamide (37.5:1 w/w acrylamide to methylenebisacrylamide) and 7–14% (w/v) sucrose, according to Reuter and Nickel-Reuter (1993). The gel and electrode buffer was Tris-boric acid (100 m*M*/73 m*M*, pH 8.6). The gel buffer contained 2 m*M* EDTA. The electrophoresis was performed in a Mini Protean II cell (Bio-Rad, Munich, Germany) for 16 h on ice at a constant voltage of 200 V. The coloured bands were cut out and pigment proteins were electroeluted at 200 V in the same electrode buffer containing 7% (w/v) sucrose using a Centrilutor-Micro-Electroeluter (3000 MWCO; Amicon, Witten, Germany). Protein extracts were used for SDS-PAGE. Some gelstrips were used for absorption and fluorescence spectral analysis.

### SDS-PAGE

The protein composition of the intact PBS and the subunit complexes were analysed on 15% (w/v) polyacrylamide gels according to Reuter and Wehrmeyer (1988). For a better resolution of the linker polypeptides, 6% (v/v) isopropanol was added to the separation gel as described by Reuter and Nickel-Reuter (1993). Electrophoresis was performed on ice in a Mini Protean II cell (Bio-Rad) for 2 h at 200 V. Samples were solubilized at 80°C for 10 min in a buffer containing 5% (w/v) SDS, 2% (w/v) dithiothreitol, 20% (w/v) sucrose, 0.25 *M* Tris-HCl, pH 6.8 and 0.02% (w/v) Coomassie Brillant Blue G250. After electrophoresis, gels were fixed for 1 h in 7% (v/v) acetic acid with 40% methanol, stained overnight in 0.025% (w/v) Coomassie blue R250 in 7% (v/v) acetic acid and destained in 7% (v/v) acetic acid.

The apparent molecular masses of polypeptides were calculated by using a broad range protein marker SDS-PAGE standard (Bio-Rad). For calculation of the apparent molecular masses and for quantification of relative protein content the stained gels were scanned at 520–570 nm with a GS-700 Imaging Densitometer (Bio-Rad) with Multi-Analyst Software (Bio-Rad).

### Absorption and fluorescence measurements

Absorption spectra were recorded with a UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). Fluorescence emission spectra were measured at 77 K and at room temperature in a spectrofluorometer (PTI; Photon Technology International, Lawrenceville, NJ, USA) with an excitation of 450 nm.

### Phycobiliprotein and protein determination

The phycobiliprotein contents were determined as described by Lüder et al. (2001). The protein contents were determined with Bio-Rad-Protein Assay (Bio-Rad).

### Electron microscopy

For fixation, the isolated PBS were immediately diluted 1:1 with isolation buffer containing 0.2% (v/v) glutaraldehyde and 15% (v/v) sucrose and stored for 2 h according to Wehrmeyer et al. (1993). They were then negatively stained with uranyl acetate and subsequently examined in a transmission electron microscope (EM 902; Zeiss, Oberkochen, Germany). The negative staining procedure was performed according to Nermut (1973) with the drop technique. 400 mesh formvar-coated grids were floated on a sample drop for 5 min, washed 3 times in distilled water and then floated again on a drop of 2% uranylacetate for 1 min. The excess solution was removed with filter paper and the grids were subsequently viewed in the microscope.

## Results

Intact undissociated PBS from the Antarctic macroalga *P. decipiens* and the temperate macroalga *P. palmata* when separated on discontinuous sucrose gradients, appeared in the 35% (w/v) sucrose layer as two discrete bands. Only one PBS band could be isolated from the unicellular red algae *P. cruentum* and *R. violacea* (Fig. 1). Dissociated PBS were found between the 15 and 35% (w/v) sucrose layers. Free carotenoids and chlorophyll were found on the top of the gradients.

PBS were proved to be intact by low and room temperature fluorescence emission spectroscopy. The emission spectra from the upper and lower banded PBS ($PBS_{up}$, $PBS_{low}$) of *P. decipiens* are shown in Fig. 2. At 77 K, the emission peaks of $PBS_{up}$ and $PBS_{low}$ were at 680 nm, which indicates an effective energy transfer to the terminal energy acceptor of the PBS. At room temperature, the emission peak of the $PBS_{up}$ was at 666 nm whereas that of $PBS_{low}$ was slightly

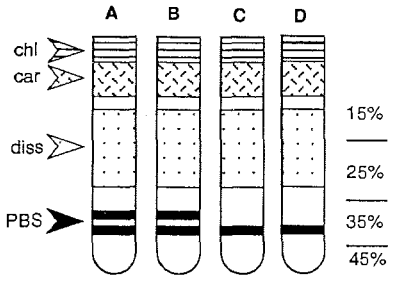


Fig. 1. Sucrose density gradients with PBS isolated from: (A) *P. decipiens*, (B) *P. palmata*, (C) *P. cruentum* and (D) *R. violacea*. PBS, intact phycobilisomes; diss, dissociated PBS; chl, chlorophyll; car, carotenoids.

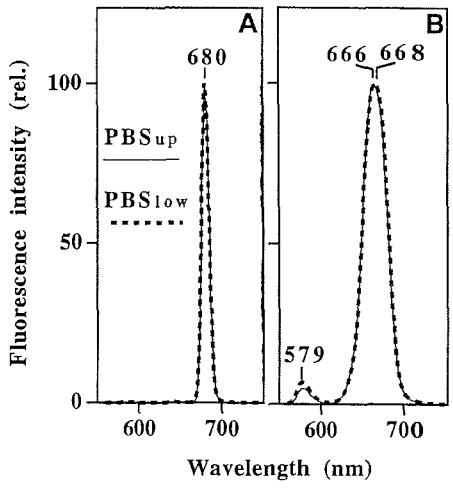


Fig. 2. Fluorescence emission spectra at 77 K (A) and at room temperature (B) of PBS ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*, excitation at 450 nm.

shifted to 668 nm. Furthermore, a small peak was evident at 579 nm in both cases.

The absorption spectra of the $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* (Fig. 3) showed no significant differences and allowed us to identify RIII-phycoerythrin (RIII-PE: 496 <

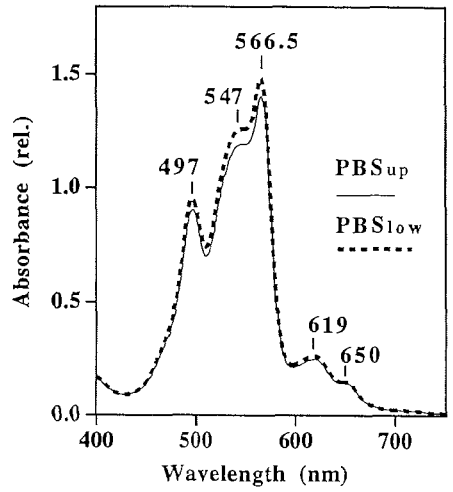


Fig. 3. Absorption spectra of PBS ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens* dissolved in 0.1 *M* K phosphate, pH 6.8.

Table 1. Ratios of phycobiliproteins in the isolated PBS from the studied species (means and SD, n = 5).

| Alga | PBS forms | Ratios of phycobiliproteins | | |
|---|---|---|---|---|
| | | PE:APC | PC:APC | PE:PC |
| *P. decipiens* (0°C culture) | $PBS_{up}$ | 12.30 (0.28) | 1.52 (0.04) | 8.08 (0.07) |
| | $PBS_{low}$ | 11.82 (0.09) | 1.50 (0.02) | 7.86 (0.06) |
| *P. palmata* (10°C culture) | $PBS_{up}$ | 7.49 (0.08) | 1.33 (0.03) | 5.64 (0.06) |
| | $PBS_{low}$ | 7.47 (0.07) | 1.28 (0.02) | 5.85 (0.07) |
| *R. violacea* (hemidiscoidal PBS) | | 13.63 (0.09) | 1.77 (0.01) | 7.70 (0.03) |
| *P. cruentum* (ellipsoidal PBS) | | 14.76 (0.23) | 1.66 (0.02) | 8.90 (0.08) |

544 < 567 nm) according to Rennis and Ford (1992). The phycobiliprotein contents and their ratios are summarized in Table 1. The ratios of PE:APC, PC:APC and PE:PC of the $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* showed no significant differences. *Palmaria palmata* had generally low ratios, but there were no significant differences between $PBS_{up}$ and $PBS_{low}$. *P. cruentum* and *R. violacea* had the highest ratios and, compared with *R. violacea*, *P. cruentum* had a higher PE:APC ratio probably as a result of their more complex hemiellipsoidal PBS.

Fig. 4 shows the phycobiliprotein subunit complexes of $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* separated by native PAGE. $PBS_{up}$ and $PBS_{low}$ were separated into one lilac (2-PC), one turquoise (6-APC) and 4 pink (1-PE, 3-PE, 4-PE and 5-PE) bands. The characteristic absorption spectra of these subunit complexes are shown in Fig. 5 and their absorption maxima are summarized in Table 2. The 4 pink coloured bands of $PBS_{up}$ and $PBS_{low}$ are PE subunit complexes. Within bands 1-PE to 5-PE the absorption maxima shifted from 538 to 544.5 nm and from 566 to 567.5 nm in $PBS_{up}$, from 534 to 544.5 nm and from 565 to 567 nm in $PBS_{low}$. Furthermore, the shape of the spectrum around 544 nm was different (1-PE: peak, 3-PE: shoulder, 4-PE: plateau, 5-PE: peak). $PBS_{up}$ and $PBS_{low}$ differed only in the 1-PE band, the band of $PBS_{low}$ showed a drastic reduction in absorbance around 544 and 567 nm, compared with the 1-PE band of $PBS_{up}$. The lilac band (2-PC) could be identified as RI-phycocyanin complex (RI-PC: 555 < 617 nm) and the turquoise band (6-APC) as allophycocyanin complex (APC: 652 nm) with PE contamination according to Glazer and Hixon (1975), Ducret et al. (1994) and Sidler (1994).

Coomassie blue staining of the native PAGE allowed quantification of the total protein content of the subunit complexes. The percentage distribution of the total PBS protein content and the PE complexes are also summarized in Table 2. There were no quantitative differences in the amount of the subunit complexes when comparing $PBS_{up}$ and $PBS_{low}$. The 5-PE subunit complex made up the largest proportion, with 42% of the total PE content, followed by the 3-PE (29%), 1-PE (21%) and 4-PE complex (8%).

The fluorescence emission spectra of the subunit complexes are shown in Fig. 6 and their emission maxima are summarized in Table 2. They give information about the energy transfer and the aggregation states. Comparing the PE complexes 1-PE to 5-PE a red-shift can be noticed, from band 1-PE to 5-PE the peak shifted to the right from 580 to 585 nm in $PBS_{up}$ and from 579 to 585 nm in $PBS_{low}$. The 3-PE complex shows a further peak at 595 nm (and slightly at 618 and 632 nm, which most probably resulted from PC contaminations). The phycocyanin complex (2-PC) had a characteristic emission peak at 640 nm, whereas the allophycocyanin complex (6-APC) showed a characteristic peak at 662 nm, but also a prominent peak at 580, which resulted from contamination with PE. Please note the extremely low fluorescence signal of the 1-PE complex compared with the others PE complexes (3-, 4- and 5-PE).

The subunit complexes and the total PBS of the $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* were further analysed by SDS-PAGE (Fig. 7), allowing the identification of the phycobiliprotein subunits and of some linker polypeptides. The apparent molecular masses of the proteins and their percentage distribution are listed in Table 3. The apparent molecular masses of the phycobiliprotein subunits ($\alpha$ and $\beta$) ranged from 16.8 to 21.6 kDa. All PE complexes had $\alpha$- and $\beta$-subunits of the same molecular masses (18.7 and 21.6 kDa, respectively) but 3 coloured $\gamma$-subunits with different molecular masses (1-PE 33.5 kDa, 3-PE: 33.5 kDa, 4-PE: 40.3 kDa, 5-PE: 37.3 kDa). The $\gamma$-subunit of 33.5 kDa of the 1-PE complex could only be detected in the $PBS_{low}$. Three low molecular mass polypeptides (5.8, 10.2 and 13.3 kDa) appeared in $PBS_{up}$, which might be disintegration products of the missing $\gamma$-subunit. Colourless linker

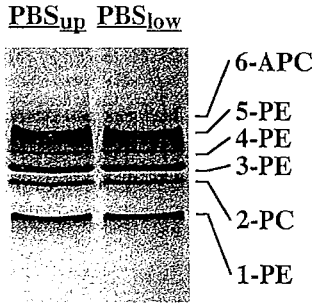


Fig. 4. Subunit complexes of PBS ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens* separated by native gradient PAGE (4–14% in 100/73 m*M* Tris boric acid, pH 8.6 and 7–14% sucrose).

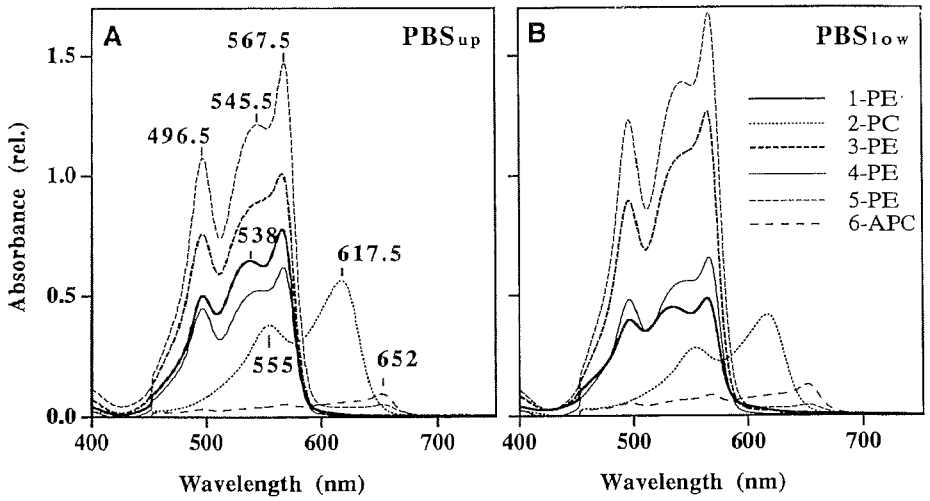


Fig. 5. Absorption spectra of the coloured subunit complexes of PBS [(A) $PBS_{up}$ and (B) $PBS_{low}$] from *P. decipiens*.

polypeptides of 10.0, 30.6 and 38.9 kDa were found. The 38.9 kDa polypeptide probably functions as PC rod linker ($L_R$) and the 30.6 kDa polypeptide as PC-APC rod-core linker ($L_{RC}$). The small 10 kDa polypeptide is the core linker ($L_C$). The polypeptide at 85.0 kDa was the largest linker polypeptide and represents the PBS anchor linker ($L_{CM}$). The polypeptides between 53.3 and 75.4 kDa were often found in SDS gels of PBS and are interpreted to be contaminants. The 53.3 kDa polypeptide is probably the large subunit of Rubisco (Marquardt et al. 1999). Polypeptides of approximately 45–50 kDa in PBS isolations were supposed to be FNR (ferredoxin:$NADP^+$ oxidoreductase) (Schluchter and Bryant 1992, Sidler 1994, Ritz et al. 1998). FNR is thought to be associated with the PBS, probably attached to the peripheral rods.

In *P. decipiens*, the phycobiliprotein subunits ($\alpha$ and $\beta$) accounted for approximately 80% and the linker polypeptides (including $\gamma$-subunits) for about 17% of the total proteins stainable with Coomassie blue. The percentage distribution of the subunits and of the linker polypeptides in the $PBS_{up}$ and $PBS_{low}$ is similar, except that the amount of the small core linker $L_C$, the large anchor linker $L_{CM}$ and the 40.3 kDa $\gamma$-subunit were somewhat higher in the $PBS_{up}$.

The absorption and fluorescence characteristics and the polypeptide composition of the subunit complexes allowed us to reconstruct aggregation states as summarized in Table 2.

Negatively stained PBS from *P. decipiens* are shown in Fig. 8. The $PBS_{up}$ and $PBS_{low}$ seem to be the same hemiellipsoidal PBS type with pure structural details. The $PBS_{up}$ have a basal length of about 57–64 nm and a height of about 35 nm. The $PBS_{low}$ seems to be somewhat smaller with a basal length of 50–57 nm and a height of about 32–35 nm.

## Discussion

### Double-banded PBS

The main result of the present work is the finding of double-banded PBS ($PBS_{up}$ and $PBS_{low}$) in *P. decipiens*. The isolation of one PBS type from the unicellular microalgae *P. cruentum* and *R. violacea* using the same protocol excludes the possibility that the double band is an extraction artefact or the result of partial proteolytic degradation. The appearance of two PBS bands seems not growth temperature specific as two PBS forms were also isolated from the temperate *P. palmata* grown at 10°C. Usually, PBS are very sensitive to low isolation temperatures. Isolation temperatures of 4–10°C result in an uncoupling of PBS or a loss of APC (Gantt et al. 1979, Mörschel and Rhiel 1987, Glazer 1988). In our preparations, an effective energy transfer to the terminal energy acceptor of the PBS was shown by low temperature fluorescence emission spectroscopy in all species. Formation of artificial PBS aggregates was found once, when PBS were isolated in the non-ionic detergent Triton X-100 from Glazer et al. (1979). But this phenomenon was not found in all studied cyanobacteria and not in red algae. Similar observations are not known from preparations with N,N-dimethyl-dodecylamine-N-oxide used in this study. Furthermore, we have indications that the two PBS forms can be seen in connection with seasonally changing light conditions. In a forthcoming study (Lüder UH, Knoetzel J and Wiencke C), we will show that *P. decipiens* changes the

Table 2. Characteristic absorption and fluorescence emission maxima and polypeptide composition of the native separated subunit complexes of PBS from *P. decipiens*. a: The PBS were separated on 1 mm native PAGE, stained with Coomassie blue and densitometrically calculated (means and SD, n = 4); c: contamination; p: plateau; s: shoulder; w: weak; *γ-subunit was dissociated in SDS-PAGE, probably.

| Band no. | Absorption maxima (nm) | Fluorescence emission maxima (nm) | % of total PBS protein[a] | % of total PE protein[a] | Identification | Possible subunit complexes |
|---|---|---|---|---|---|---|
| $PBS_{up}$ | | | | | | |
| 1-PE | 497–538–566 | 580 | 17.1 (0.9) | 20.9 (1.0) | r-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_3\ (\gamma^{33.5})^*$ |
| 2-PC | 555–617.5 | 638 | 11.9 (0.4) | — | R-phycocyanin | $(\alpha^{PC}\ \beta^{PC})_3\ L^{38.9}$ |
| 3-PE | 496.5–544.5$^s$–566 | 582–595–618$^w$–632$^w$ | 23.8 (0.8) | 29.1 (1.2) | R-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_6\ \gamma^{33.5}$ |
| 4-PE | 496.5–544.5$^p$–567 | 582 | 6.6 (1.0) | 8.1 (1.2) | R-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_6\ \gamma^{40.3}$ |
| 5-PE | 496.5–544.5–567.5 | 585 | 34.2 (1.0) | 41.9 (1.2) | R-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_6\ \gamma^{37.3}$ |
| 6-APC | 652 | 580$^c$–662 | 6.4 (0.4) | — | allophycocyanin | $(\alpha^{APC}\ \beta^{APC})_3\ L_c^{10}$ |
| $PBS_{low}$ | | | | | | |
| 1-PE | 497–534–565 | 579 | 17.4 (2.2) | 21.2 (2.3) | r-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_3\ \gamma^{33.5*}$ |
| 2-PC | 555–617.5 | 640 | 11.8 (1.6) | — | R-phycocyanin | $(\alpha^{PC}\ \beta^{PC})_3\ L_R^{38.9}$ |
| 3-PE | 496.5–544.5$^s$–565.5 | 581–593–618$^w$–632$^w$ | 23.5 (0.8) | 28.8 (1.5) | R-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_6\ \gamma^{33.5}$ |
| 4-PE | 496.5–544.5$^p$–566.5 | 581 | 6.3 (0.9) | 7.7 (1.0) | R-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_6\ \gamma^{37.3}$ |
| 5-PE | 496.5–544.5–567 | 585 | 34.6 (0.8) | 42.3 (1.6) | R-phycoerythrin | $(\alpha^{PE}\ \beta^{PE})_6\ \gamma^{40.3}$ |
| 6-APC | 652 | 575$^s$–663 | 6.5 (0.4) | — | allophycocyanin | $(\alpha^{APC}\ \beta^{APC})_3\ L_c^{10}$ |

amounts of the $PBS_{up}$ and $PBS_{low}$ during a 1-year cycle under fluctuating Antarctic day-lengths and that under an extended dark period the $PBS_{low}$ form completely disappears. This supports the conclusion that the two PBS forms are no artificial aggregates or dissociation products. Instead, we assume that two PBS forms have a physiological function and might be advantageous for *P. decipiens* to acclimate to seasonal light conditions.

The presence of two PBS types has been reported before only for two *Porphyra* species and not for unicellular algae. Algarra et al. (1990) observed two kinds of well-coupled (ellipsoidal and hemidiscoidal) PBS in *Porphyra umbilicalis* collected from the field. Shi et al. (1995) observed two types of PBS during the life cycle of *Porphyra yezoensis*. In two species of cyanobacteria belonging to the *Phormidium* genus the presence of hemidiscoidal and hemiellipsoidal PBS, according to the light quality, was observed (Westermann et al. 1993, Westermann and Wehrmeyer 1995). In the above studies, the authors found considerable differences with respect to the phycobiliprotein composition and the structure of the two PBS types and they also suggested an ecophysiological function.

### The phycobiliproteins and their chromophores

The present study on *P. decipiens* and *P. palmata* reveals no evidence for quantitative differences between the $PBS_{up}$ and $PBS_{low}$ in the absorption properties (Fig. 3) and in the phycobiliprotein ratios (PE:APC, PC:APC and PE:PC; Table 1).

The identification of the phycobiliproteins RIII-PE, RI-PC and APC in *P. decipiens* extend the results of Czeczuga (1985) on the same species. We have further identified RII-PE in *P. palmata* and B-PE in *P. cruentum* and in *R. violacea* (data not shown), which support previous studies by Honsell et al. (1984) and Rennis and Ford (1992). The R-PE type has 3 variants (RI-III) and is widespread within the Florideophyceae (Honsell et al. 1984, Rennis and Ford 1992). The 3 R-PE variants show slight spectral differences caused by the various chromophore compositions of their phycobiliproteins (Stadnichuk 1995). Recently a novel R-PE, called R-PE IV, was discovered in the Antarctic red alga *Phyllophora antarctica* (MacColl et al. 1996, 1999).

### The r-PE-subunit complex

Almost no differences in the subunit and polypeptide composition between the $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* were detectable (Tables 2 and 3). However, the low molecular mass PE subunit complex (1-PE complex) of $PBS_{low}$ separated by native PAGE (Fig. 4) showed drastically reduced absorbances at 534 nm and at 565 nm (Fig. 5), although the existence of all 3 PE-subunits ($\alpha$, $\beta$ and $\gamma$) was supported by SDS-PAGE (Fig. 7). Conversely, the 1-PE complex of $PBS_{up}$ showed a 'normal' PE absorption spectrum, but in the SDS-PAGE no $\gamma$-subunit was found and 3 low molecular weight polypeptides appeared, probably dissociation products of the missing $\gamma$-subunit. We assume a weaker stability of the $\gamma$-subunit, which is important for the incorporation of PE in the PBS, in the 1-PE complex of the $PBS_{up}$, relative to $PBS_{low}$.

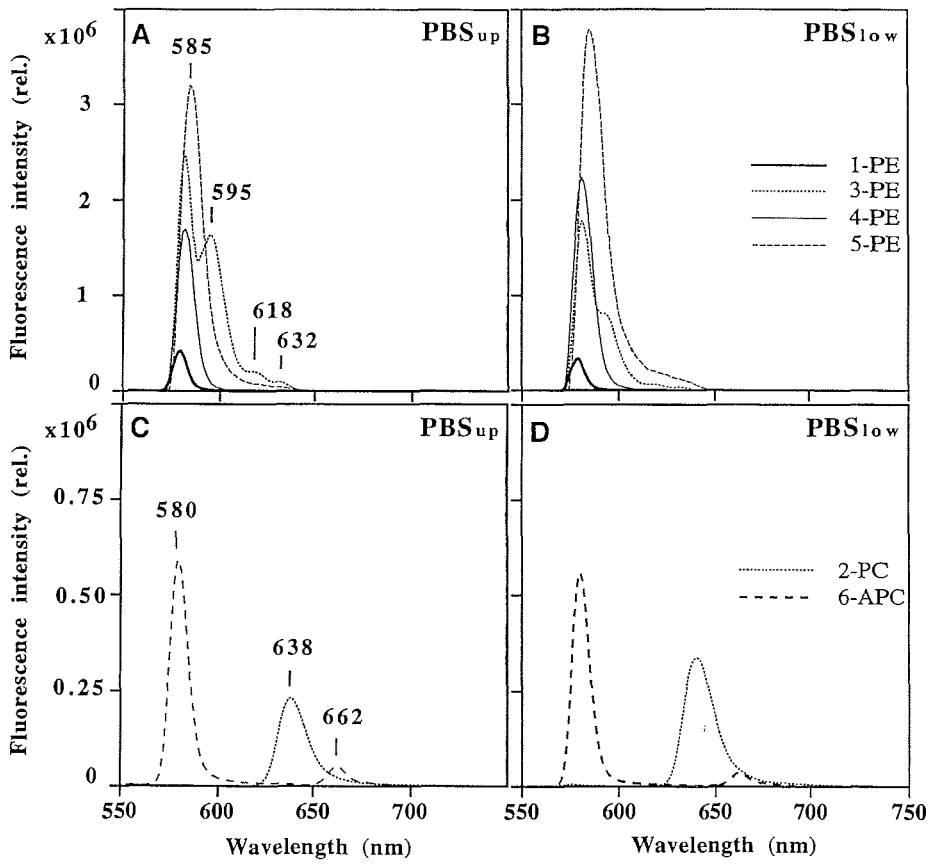


Fig. 6. Fluorescence emission spectra at 77 K (excitation at 450 nm) of the coloured subunit complexes of PBS [(A, C) $PBS_{up}$ and (B, D) $PBS_{low}$] from *P. decipiens*.

Consequently, the $PBS_{up}$ may be a little more loosely constructed.

In both PBS forms ($PBS_{up}$ and $PBS_{low}$), the 1-PE complex made up about 21% of the total PE content (Table 2), but showed a very low fluorescence signal (Fig. 6). Consequently, it could belong to the so-called r-PE. This r-PE was previously described in *Griffithsia pacifica* (Gantt and Lipschultz 1980) and in *Audouinella saviana* (Talarico 1990). It forms aggregates of monomers, dimers, trimers or tetramers and has a low molecular mass of only 55–110 kDa compared with R-PE with 265 kDa, which is suspected to form two hexameric aggregates. R-PE and r-PE occurred in a ratio of 4:1 and the quantum yield of r-PE is only one-third as great as that of R-PE. It is comparable with b-PE found in *Porphyridium cruentum* (Gantt and Lipschultz 1974, Glazer and Hixon 1977). The functions of these r- and b-PEs are still unclear (Sidler 1994, Talarico 1996). It has been suggested that b-PE is somewhat less stable than B-PE when released from PBS (Gantt 1990). It is thought that they mediate a closer packing of biliproteins within the PBS (Gantt and Lipschultz 1974, 1980). Dubinsky (1992) further suggested that this 'package effect' may help to dissipate excess energy. Bird et al. (1982) found a nitrogen reserve in these r- and b-PEs. It can also be the precursor used to build up larger hexameric functional subunits when a rapid PBS formation is needed (Talarico 1990, 1996). A closer packing of the phycobiliproteins within the $PBS_{low}$ from *P. decipiens* would also explain why no differences in the ratios of the phycobiliproteins (Table 1) were found, but a smaller size of the negatively stained $PBS_{low}$ (Fig. 8) was measured.

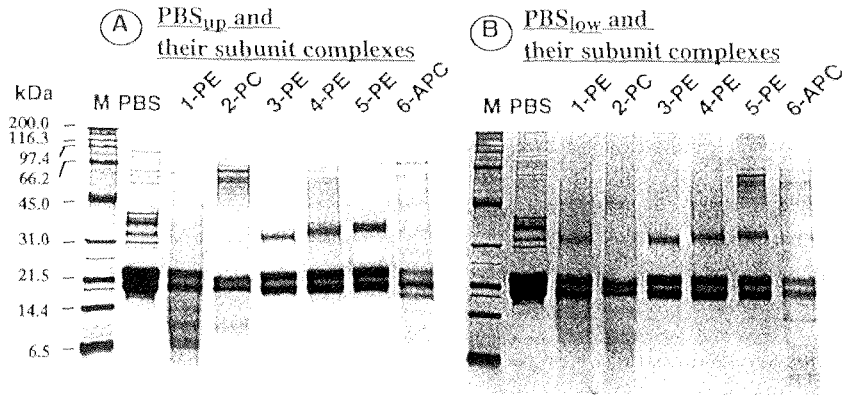


Fig. 7. Polypeptide composition of PBS [(A) $PBS_{up}$ and (B) $PBS_{low}$] isolated from *P. decipiens* and their subunit complexes, separated by 15% SDS-PAGE.

### The three γ-subunits

In our study, we separated 4 PE complexes by native PAGE (Fig. 4), with the low molecular mass 1-PE complex probably being r-PE. The remaining 3 high molecular mass PE complexes (3-, 4- and 5-PE) differ in their absorption characteristics around 544 nm (Fig. 5) probably because of different chromophore molar ratios in the 3 γ-subunits detected by SDS-PAGE (Fig. 7). Similar observations were made in *Aglaothamnion neglectum* by Apt et al. (1993). These authors isolated two γ-subunits ($\gamma_1$, $\gamma_2$) with distinct molecular masses and different chromophore ratios of phycoerythrobilin:phycourobilin (PEB:PUB) and showed that each subunit complex [$(\alpha\beta)_6\gamma_1$, $(\alpha\beta)_6\gamma_2$] exhibited a different spectral pattern at 550 nm. Accordingly, we assume that in *P. decipiens* the γ-subunit of the 3-PE complex has a higher ratio of PEB:PUB, 4-PE an equal ratio of PEB:PUB and 5-PE a lower ratio of PEB:PUB. These should be proved by reverse-phase high-pressure liquid chromatography as described by Swanson and Glazer (1990) and Stadnichuk et al. (1993). The γ-subunits of red algae generally carry 4 or 5 phycobilins (PUB and/or PEB) and the number of γ-subunits varies within species (Stadnichuk 1995, Stadnichuk et al. 1997, Ritz et al. 1998, Talarico et al. 1998). Only a single γ-subunit was identified in R-PE from *P. palmata* (Galland-Irmouli et al. 2000).

What are the advantages of different γ-subunits? Our results showed that the 3 γ-subunits in *P. decipiens* induced a minor red-shift in the absorption and fluorescence maxima

Table 3. Polypeptides of the PBS from *P. decipiens* (means and SD, n = 6). a: Densitometrically calculated from the area under peaks of Coomassie blue stained SDS gel scans. b: Chromphore containing polypeptides visible under UV light before Coomassie blue staining.

| Band no. | Mol. mass (kDa) | % of total PBS protein[a] | | Identification | |
|---|---|---|---|---|---|
| | | $PBS_{up}$ | $PBS_{low}$ | | |
| 1 | 99.0 (2.3) | 0.46 (0.05) | 0.64 (0.07) | ? | |
| 2[b] | 85.0 (1.3) | 1.56 (0.09) | 1.41 (0.11) | $L_{CM}$ | anchor linker |
| 3 | 75.4 (1.2) | 0.53 (0.10) | 0.53 (0.21) | ? | |
| 4 | 65.3 (2.1) | 0.65 (0.10) | 0.52 (0.04) | ? | |
| 5 | 60.7 (0.4) | 0.90 (0.14) | 0.84 (0.14) | ? | |
| 6 | 56.6 (1.3) | 0.87 (0.06) | 0.67 (0.20) | ? | |
| 7 | 53.3 (0.4) | 0.79 (0.10) | 0.75 (0.16) | ? | |
| 8[b] | 40.3 (0.2) | 2.89 (0.42) | 2.39 (0.31) | $\gamma^{PE}$ | PE γ-subunit |
| 9 | 38.9 (0.3) | 1.72 (0.23) | 1.75 (0.21) | $L_R$ | PC linker |
| 10[b] | 37.3 (0.3) | 4.77 (0.23) | 4.61 (0.40) | $\gamma^{PE}$ | PE γ-subunit |
| 11[b] | 33.5 (0.3) | 2.87 (0.13) | 2.85 (0.19) | $\gamma^{PE}$ | PE γ-subunit |
| 12 | 30.6 (0.3) | 2.28 (0.20) | 2.14 (0.07) | $L_{RC}$ | PC-APC linker |
| 13[b] | 21.6 (0.5) | * | * | $\beta^{PE}$ | PE β-subunit |
| 14[b] | 21.4 (0.4) | * | * | $\beta^{PC}$ | PC β-subunit |
| 15[b] | 19.4 (0.4) | *together = | *together = | $\alpha^{PC}$ | PC α-subunit |
| 16[b] | 19.2 (0.5) | 78.87 (1.17) | 80.17 (1.50) | $\beta^{APC}$ | APC β-subunit |
| 17[b] | 18.7 (0.5) | * | * | $\alpha^{PE}$ | PE α-subunit |
| 18[b] | 16.8 (0.2) | * | * | $\alpha^{APC}$ | APC α-subunit |
| 19 | 10.0 (0.2) | 0.72 (0.13) | 0.59 (0.18) | $L_C$ | core linker |

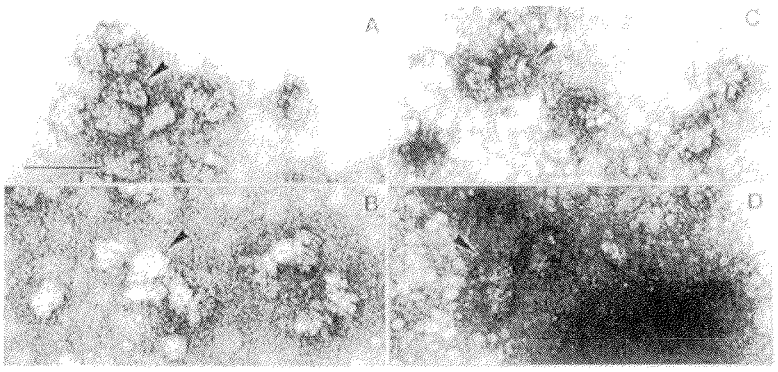


Fig. 8. Negatively stained isolated PBS [(A, B) $PBS_{up}$ and (C, D) $PBS_{low}$] from *P. decipiens*. Arrows indicate hemiellipsoidal PBS forms. Scale bar = 0.1 μm.

of the native separated PE subunit complexes (Table 2). These minimal changes might support the unidirectional transfer of excitation energy in the PBS from the periphery to the core. Besides their structural function, linker polypeptides are known to be involved in energy transfer. They modulate the spectral properties of the phycobiliprotein trimers and hexamers by interacting with the chromophores or indirectly by changing the chromophore environment (Glazer 1985, Mimuro et al. 1986, Watson et al. 1986, Glazer and Clark 1986).

Furthermore, 3 γ-subunits resulting in 3 different PE-subunit complexes [$(\alpha\beta)_6\gamma_1$, $(\alpha\beta)_6\gamma_2$, $(\alpha\beta)_6\gamma_3$] might increase the variability of rod length in *P. decipiens* and may be advantageous for a quick variation in PBS size in order to react to rapid environmental changes. Previous studies showed that a loss of the terminal PE hexamers was accompanied by a loss of its associated γ-subunit, observed under high light conditions in *R. violacea* (Bernard et al. 1996). Such a loss would also explain how the PEB:PUB ratio of R-PE can be modulated by varying the light intensity during growth (Yu et al. 1981). Furthermore, in a mutant of *Porphyridium* sp. one of the 3 γ-subunits was missing and two types of rods were probably present, one containing only PE and the other containing PE bound to PC (Ritz et al. 1998). Apt et al. (1993) showed that the level of transcripts encoding the γ-subunit changes in different environmental conditions. So, the PBS size (rod length) may be modulated by the transcription of γ-subunits. We know that *P. decipiens* changes the phycobiliprotein ratios and probably the PBS size during seasonally changing light conditions (Lüder et al. 2001); therefore, it would be very interesting to observe the expression of the 3 γ-subunits under different light conditions.

We conclude that the two forms of PBS ($PBS_{up}$ and $PBS_{low}$) in *P. decipiens* represent two aggregation states of different stability, which might allow a rapid reaction to environmental changes of light intensity as previously discussed by Algarra et al. (1990). The presence of 3 γ-subunits resulting in 3 different PE-subunit complexes might increase the unidirectional energy transfer and hence might modulate the PBS size. Nevertheless, the unexpected finding of two PBS forms in macroalgae shows the need to investigate PBS of macroalgae in more detail. In nature, macroalgae are fixed to the substratum and have to adjust to environmental conditions much more than microalgae, which are able to move in the water column. Therefore, many macroalgae might develop different strategies to aid in the acclimation to environmental changes in light.

*Acknowledgements* – This paper is dedicated to Prof. Dr Werner Wehrmeyer, University of Marburg on the occasion of his 70th birthday. This work was supported by grants from the Deutsche Forschungsgemeinschaft. We gratefully thank E. Lorenz and U. Humbolt for excellent technical support and Prof. Grimme for laboratory space. We acknowledge J. Marquardt for technical and intellectual input/support. Thanks go to M. Schoenwaelder for critically reading the manuscript.

## References

Algarra P, Thomas JC, Mousseau A (1990) Phycobilisome heterogeneity in the red alga *Porphyra umbilicalis*. Plant Physiol 92: 570–576

Apt KE, Hoffman NE, Grossman AR (1993) The γ-subunit of R-phycoerythrin and its possible mode of transport into the plastid of red algae. J Biol Chem 268: 16208–16215

Bernard C, Etienne A-L, Thomas J-C (1996) Synthesis and binding of phycoerythrin and its associated linkers to the phycobilisome in *Rhodella violacea* (Rhodophyta): Compared effects of high light and translation inhibitors. J Phycol 32: 265–271

Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J Phycol 18: 344–348

Czeczuga B (1985) Light-harvesting phycobiliprotein pigments of the red alga *Leptosomia simplex* from the Antarctic. Polar Biol 4: 179–181

Dubinsky Z (1992) The functional and optical absorption cross sections of phytoplankton photosynthesis. In: Falkowski PG, Woodhead AD (eds) Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York, NY, pp 31–45. ISBN 0-306-44192-6

Ducret A, Sidler W, Frank G, Zuber H (1994) The complete amino acid sequence of R-phycocyanin-I α and β subunits from the red alga *Porphyridium cruentum*. Structural and phylogenetic relationships of the phycocyanins within the phycobiliprotein families. Eur J Biochem 221: 563–580

Galland-Irmouli AV, Pons L, Luçon M, Villaume C, Mrabet NT, Guéant JL, Fleurence J (2000) One step purification of R-phycoerythrin from the red macroalga *Palmaria palmata* using preparative polyacrylamide gel electrophoresis. J Chromatog B 739: 117–123

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of the Red Algae. Cambridge University Press, Cambridge, pp 203–219. ISBN 0-521-34301-1

Gantt E, Lipschultz CA (1974) Phycobilisomes of *Porphyridium cruentum*: Pigment analysis. Biochemistry 13: 2960–2966

Gantt E, Lipschultz CA (1980) Structure and phycobiliprotein composition of phycobilisomes from *Griffithsia pacifica* (Rhodophyceae). J Phycol 16: 394–398

Gantt E, Lipschultz CA, Grabowski J, Zimmerman BK (1979) Phycobilisomes from blue-green and red algae. Isolation and dissociation characteristics. Plant Physiol 63: 615–620

Glazer AN (1985) Phycobilisomes: Structure and dynamics of energy flow. In: Steinback KE, Bonitz S, Arntzen CJ, Bogorad L (eds) Molecular Biology of the Photosynthetic Apparatus. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, pp 231–240. ISBN 0-87969-188-3

Glazer AN (1988) Phycobilisomes. Methods Enzymol 167: 304–312

Glazer AN, Clark JH (1986) Phycobilisomes. Macromolecular structure and energy flow dynamics. Biophys J 49: 115–116

Glazer AN, Hixon CS (1975) Characterization of R-phycocyanin. Chromophore content of R-phycocyanin and C-phycoerythrin. J Biol Chem 250: 5487–5495

Glazer AN, Hixon CS (1977) Subunit strncture and chromophore composition of rhodophytan phycoerythrins. *Porphyridium cruentum* B-phycoerythrin and b-phycoerythrin. J Biol Chem 252: 32–42

Glazer AN, Williams RC, Yamanaka G, Schachman HK (1979) Characterisation of cyanobacterial phycobilisomes in zwitterionic detergents. Proc Natl Acad Sci USA 76: 6162–6166

Holzwarth AR (1991) Structure-function relationships and energy transfer in phycobiliprotein antennae. Physiol Plant 85: 518–528

Honsell E, Kosovel V, Talarico L (1984) Phycobiliprotein distribution in Rhodophyta: Studies and interpretations on the basis of their absorption spectra. Bot Mar 27: 1–6

Jones RF, Speer HL, Kury W (1963) Studies on the growth of the red alga *Porphyridum cruentum*. Physiol Plant 16: 636–643

Lüder UH, Knoetzel J, Wiencke C (2001) Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. Polar Biol (in press)

MacColl R (1998) Cyanobacterial phycobilisomes. J Struct Biol 124: 311–334

MacColl R, Eisele LE, Williams EC, Bowser SS (1996) The discovery of a novel R-phycoerythrin from an Antarctic red alga. J Biol Chem 271: 17157–17160

MacColl R, Eisele LE, Malak H, Endres RL, Williams EC, Bowser SS (1999) Studies on R-phycoerythrins from two Antarctic marine red algae and a mesophilic red alga. Polar Biol 22: 384–388

Marquardt J, Schultze A, Rosenkranz V, Wehrmeyer W (1999) Ultrastructure and photosynthethic apparatus of *Rhodella violacea* (Porphyridiales, Rhodophyta) grown under iron-deficient conditions. Phycologia 38: 418–427

Mimuro M, Rümbeli R, Flüglistaller P, Zuber H (1986) The microenvironment around the chromophores and its changes due to the association states in C-phycocyanin isolated from the cyanobacterium *Mastigocladus laminosus*. Biochim Biophys Acta 851: 447–456

Mörschel E (1991) The light-harvesting antennae of cyanobacteria and red algae. Photosynthetica 25: 137–144

Mörschel E, Rhiel E (1987) Phycobilisomes and thylakoids: The light-harvesting system of cyanobacteria and red algae. In: Harris JR, Horne RW (eds) Membranous Structures, Electron Microscopy of Proteins, Vol. 6. Academic Press, London, pp 210–254. ISBN 0-12327606-3

Nermut MV (1973) Methods for negative staining. In: Schimmel G, Vogel W (eds) Methodensammlung der Elektronenmikroskopie. Wissenschaftliche Verlagsgesellschaft, Stuttgart. ISBN 3-8047-0684-3 Part 3.1.2.3

Nies M, Wehrmeyer W (1980) Isolation and biliprotein characterization of phycobilisomes from the thermophilic cyanobacterium *Mastigocladus laminosus* Cohn. Planta 150: 330–337

Rennis DS, Ford TW (1992) A survey of antigenic differences between phycoerythrins of various red algal (Rhodophyta) species. Phycologia 31: 192–204

Reuter W, Müller C (1993) Adaptation of the photosynthetic apparatus of cyanobacteria to light and $CO_2$. J Photochem Photobiol B: Biol 21: 3–27

Reuter W, Nickel-Reuter C (1993) Molecular assembly of the phycobilisomes from the cyanobacterium *Mastigocladus laminosus*. J Photochem Photobiol B: Biol 18: 51–66

Reuter W, Wehrmeyer W (1988) Core substructure in *Mastigocladus laminosus* phycobilisomes. I. Microheterogeneity in two of three allophycocyanin core complexes. Arch Microbiol 150: 534–540

Reuter W, Wehrmeyer W (1990) Core substructure in *Mastigocladus laminosus* phycobilisomes. II. The central part of the tricylindrical core – $AP_{CM}$ – contains the "anchor" polypeptide and no allophycocyanin B. Arch Microbiol 153: 111–117

Ritz M, Lichtlé C, Spilar A, Joder A, Thomas J-C, Etienne A-L (1998) Characterization of phycocyanin-deficient phycobilisomes from a pigment mutant of *Porphyridium* sp. (Rhodophyta). J Phycol 34: 835–843

Schluchter WM, Bryant DA (1992) Molecular characterization of ferredoxin-NAPD+ oxidoreductase in cyanobacteria: cloning and sequence of the petH gene of *Synechoccus* sp PCC 7002 and studies on the gene product. Biochemistry 31: 3092–3102

Shi DJ, Peng GH, Chen JX, Wang HJ, Jin P, Feng LJ, Zhang H, Fei XG, Tseng CK, Tang PS (1995) Possible occurrence and characteristics of two types of phycobilisomes during ontogenesis of red alga *Porphyra yezoensis*. In: Mathis P (ed) Photosynthesis: From Light to Biosphere, Vol. I. Kluwer Academic Publishers, Dordrecht, pp 383–386. ISBN 0-7923-3862-6

Sidler WA (1994) Phycobilisome and phycobiliprotein structures. In: Bryant AD (ed) The Molecular Biology of Cyanobacteria. Klnwer Academic Publishers, Dordrecht, pp 139–216. ISBN 0-7923-3222-9

Stadnichuk IN (1995) Phycobiliproteins: determination of chromophore composition and content. Phytochem Anal 6: 281–288

Stadnichuk IN, Khokhlachev AV, Tikhonova YV (1993) Polypeptide gamma-subunits of R-phycoerythrin. J Photochem Photobiol B: Biol 18: 169–175

Stadnichuk IN, Karapetyan NV, Kislov LD, Semenenko VE, Veryasov MB (1997) Two gamma-polypeptides of B-phycoerythrin from *Porphyridium cruentum*. J Photochem Photobiol B: Biol 39: 19–23

Swanson RW, Glazer AN (1990) Separation of phycobiliprotein subunits by reverse-phase high-pressure liquid chromatography. Anal Biochem 188: 295–299

Talarico L (1990) R-phycoerythrin from *Audouinella saviana* (Nemaliales, Rhodophyta). Ultrastructural and biochemical analysis of aggregates and subunits. Phycologia 29: 292–302

Talarico L (1996) Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. Sci Mar 60: 205–222

Talarico L, Rascio N, Dalla Vecchia F, Maranzana G (1998) Some observations on phycobilisomes of *Pterocladiella capillacea* (Gelidiaceae, Rhodophyta). Plant Biosyst 132: 87–96

van Thor JJ, Mullineaux CW, Matthijs HCP, Hellingwerf KJ (1998) Light harvesting and state transitions in cyanobacteria. Bot Acta 111: 430–443

Watson BA, Waaland SD, Waaland JR (1986) Phycocyanin from the red alga *Anotrichium tenue*: Modification of properties by a colorless polypeptide (M 30 000). Biochemistry 25: 4583–4587

Wehrmeyer W, Mörschel E, Vogel K (1993) Core substructure in phycobilisomes of red algae. II. The central part of the tricylindrical core – $AP_{CM}$ – a constituent of hemidiscoidal phycobilisomes of *Rhodella violacea*. Eur J Cell Biol 60: 203–209

Westermann M, Wehrmeyer W (1995) A new type of complementary chromatic adaptation exemplified by *Phormidium* sp. C86: changes in the number of peripheral rods and in the stoichiometry of core complexes in phycobilisomes. Arch Microbiol 164: 132–141

Westermann M, Reuter W, Schimek C, Wehrmeyer W (1993) Presence of both hemidiscoidal and hemiellipsoidal phycobilisomes in a *Phormidium* species (Cyanobacteria). Z Naturforsch 48c: 28–34

Wiencke C (1996) Recent advances in the investigation of Antarctic macroalgae. Polar Biol 16: 231–240

Yu MH, Glazer AN, Spencer KG, West JA (1981) Phycoerythrins of the red alga *Callithamnion*. Plant Physiol 68: 482–488

Edited by W. S. Chow

## *Publication 2*

Lüder UH, Knoetzel J and Wiencke C:

# Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*.

Polar Biology (2001) 24: 598-603

Polar Biol (2001) 24: 598–603
DOI 10.1007/s003000100260

ORIGINAL PAPER

Ulrike H. Lüder · Jürgen Knoetzel · Christian Wiencke

# Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*

Accepted: 19 March 2001 / Published online: 11 May 2001


**Abstract** The influence of seasonally fluctuating Antarctic daylengths on the photosynthetic apparatus of *Palmaria decipiens* was studied in culture experiments. Maximal photosynthetic activity (ETRmax) and maximal quantum yield (Fv/Fm), measured by in vivo chlorophyll fluorescence, and concentrations of pigments (phycobiliproteins and Chl *a*) were determined monthly. Fv/Fm remained constantly high between 0.62 and 0.67 during mid-autumn, winter and spring. ETRmax and pigment contents increased continuously in mid-autumn and winter and were highest in spring. A positive correlation between pigments and ETRmax was found. In summer, ETRmax, Fv/Fm and pigment levels decreased to their lowest values. *P. decipiens* acclimated by increasing phycobilisome (PBS) number and changing PBS structure, probably changing rod length and rod number. The data show that *P. decipiens* is efficiently adapted to the short period of favourable light conditions in the field. A photoperiodic control of pigment synthesis triggered by daylength is suggested.

## Introduction

The environment of Antarctic benthic macroalgae was recently reviewed by Wiencke (1996). In the Antarctic sublittoral, algae are exposed to constant water temperatures and high nutrient concentrations over the entire year. Therefore, the seasonal development of Antarctic macroalgae depends mainly on the seasonal variation of daylengths varying, for example, between 5 h in winter and 20 h in summer at King George Island, South Shetlands. The underwater light climate is further affected by ice cover in winter and dim light or darkness prevail in the sublittoral. In summer, the water becomes turbid due to plankton blooms and glacial melt-water. So, optimal light conditions for sublittoral macroalgae are present only for a short time in the Antarctic spring, after break-up of sea ice. At this time the water is very clear and the sunlight penetrates deeply into the water column.

How do Antarctic macroalgae acclimate to seasonal changes in light? *Palmaria decipiens* is an excellent subject for this type of study, as there have been several previous studies on its life strategy. The alga is pseudoperennial and develops new blades during late winter/early spring even in darkness (Wiencke 1990b; Weykam et al. 1997). Growth starts in July and is, like photosynthesis, maximal in spring (October/November) (Wiencke 1990b; Weykam and Wiencke 1996). The light requirements for growth of this species are very low (Wiencke 1990b). *P. decipiens* even survives a period of 6 months of darkness by utilising stored floridean starch (Weykam et al. 1997).

In this study, we investigate the acclimation of the photosynthetic apparatus to seasonally changing light conditions. The algae were cultivated under seasonally fluctuating Antarctic daylengths. Photosynthetic rates and pigment contents, in particular the content of the major light-harvesting pigments – the phycobiliproteins – were determined monthly. The results give insight into the life strategy of this alga from a physiological point of view.

## Materials and methods

### Algal material and culture conditions

Tetraspores of *P. decipiens* (Reinsch) Ricker were collected from King George Island (South Shetland Islands, Antarctica) and since

This paper presents results of the Midterm Symposium of the SCAR programme "Ecology of the Antarctic Sea Ice Zone" (EASIZ). The manuscript was edited by W. Arntz and A. Clarke.

U.H. Lüder (✉) · C. Wiencke
Alfred Wegener Institute for Polar and Marine Research,
Am Handelshafen 12, 27570 Bremerhaven, Germany
E-mail: ulueder@awi-bremerhaven.de
Tel.: +49-471-48311522
Fax: +49-471-48311425

J. Knoetzel
Institute of Cell Biology, Biochemistry and Biotechnology,
University of Bremen, 28334 Bremen, Germany

then cultivated in the laboratory under seasonally fluctuating daylengths (Fig. 1A) mimicking field conditions (Wiencke 1990a, b). Numerous young gametophytes were grown at 0 ± 1°C in twenty-four 3-l glass beakers for 2 years under simulated Antarctic daylength conditions (Fig. 1A) and a constant photon fluence rate of 25 µmol photons $m^{-2}$ $s^{-1}$ using daylight fluorescent tubes (Osram L58/W19). The cultures were grown in Provasoli-enriched North Sea water (Provasoli 1966, 34‰, pH 8), aerated with pressed air. To ensure sufficient nutrient supply, the culture medium was changed every 2nd week. After about 1 year we started, under Antarctic March conditions, to harvest individual plants from different glass beakers at monthly intervals for the photosynthesis and pigment measurements.

### Chlorophyll fluorescence measurements

In vivo chlorophyll fluorescence was measured with a pulse-amplitude modulation fluorometer (PAM 2000, Walz, Germany) connected to a personal computer. All measurements were conducted in seawater at 0°C using eight to ten different individuals. Generally, the results of fluorescence measurements are consistent with those of oxygen measurements, as shown earlier (Hanelt 1992; Hanelt et al. 1992). The ratio of variable to maximal fluorescence (Fv/Fm) was determined in dark-acclimated plants as described by Bischof et al. (1999) and used as an indicator for maximal quantum yield. The maximal electron transport rate (ETRmax) was determined by ETR versus irradiance curves as described by Bischof et al. (1999) and used as an indicator of maximal photosynthetic activity (Pmax).

### Pigment determinations

Phycobiliprotein levels were determined after Rosenberg (1981) in six different individuals. The algal material was disrupted by grinding in liquid nitrogen, and extracted in 0.1 M potassium phosphate buffer, pH 6.8. The extract was frozen and thawed several times to optimise the extraction process. The resulting extract was centrifuged and the absorbance of the supernant at 565 nm, 615 nm and 650 nm was determined and calculated using the following equations:

PE(phycoerythrin)(µg/ml)

$$= 123.5 * OD565 - 73.5 * OD615 - 16.3 * OD650$$

PC(phycocyanin)(µg/ml) = 163.2 * OD615 − 117.1 * OD650

and

APC(allophycocyanin)(µg/ml) = 165.6 * OD650 − 16.4 * OD615

Chlorophyll *a* (Chl *a*) was extracted in *N,N*-dimethylformamide (DMF) according to Inskeep and Bloom (1985) and carried out with six to eight different individuals.

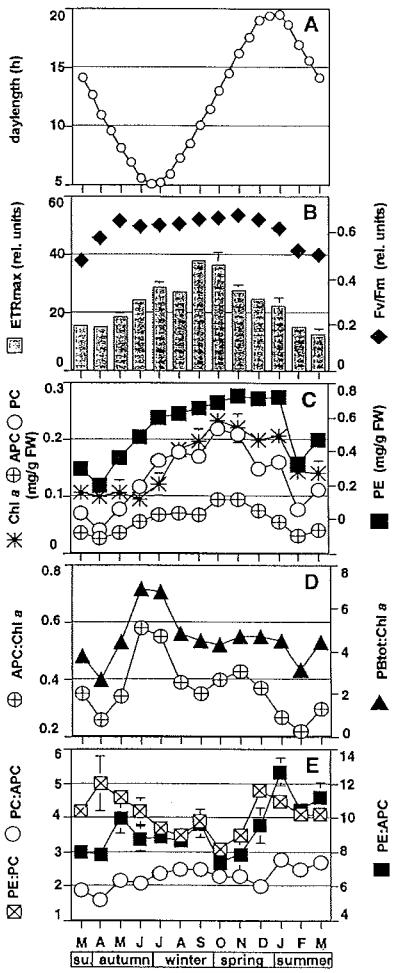


Fig. 1 Simulation of seasonal changes of daylength on King George Island, Antarctica (A). Seasonal changes in maximal electron transport rate (*ETRmax*) and in maximal quantum yield (*Fv/Fm*) (B), pigment contents (C), ratios of total phycobiliprotein and APC content to Chl *a* (D) and ratios of phycobiliprotein contents (E) (*Chl a* chlorophyll *a*, *APC* allophycocyanin, *PC* phycocyanin, *PE* phycoerythrin, *PBtot* total phycobiliprotein content). Means ± SD, $n = 8$–10 in B; $n = 6$–8 in C and E. In those cases where no error bars are given, the SD is smaller than the symbol for the mean value

## Results

*P. decipiens* was cultivated for 1 year under seasonally fluctuating daylengths according to the conditions on King George Island, Antarctica (Fig. 1A). The maximal electron transport rate increased continuously during mid-autumn and winter (May/August) and was maximal in spring (September/October) (Fig. 1B). Lowest ETRmax values were found in summer between February and April. The maximal quantum yield Fv/Fm remained high, between 0.62 and 0.67 during mid-autumn, winter and spring, decreasing from January onwards to a value of 0.47–0.50 in March, and increasing again until May (Fig. 1B).

Seasonal changes of the light-harvesting pigments were calculated per fresh weight (FW) and are shown in Fig. 1C. All three phycobiliproteins (allophycocyanin APC, phycocyanin PC and phycoerythrin PE) started to increase in May, in parallel with ETRmax, but reached maximal values slightly later in October/November (234 µg Chl *a*, 95 µg APC, 211 µg PC and 732 µg PE per g FW). Chlorophyll *a* started to increase later in July. Lowest pigment concentrations were found in summer between February and April (99 µg Chl *a*, 31 µg APC, 71 µg PC and 202 µg PE per g FW). In autumn, winter and early spring (April/October), a positive correlation was found between ETRmax and the total phycobiliprotein levels (Fig. 2A), whereas between ETRmax and Chl *a* the correlation was weaker (Fig. 2B).

The seasonal changes in the ratios of total phycobiliproteins (PBtot) to Chl *a* (PBtot:Chl *a*) are very similar to the changes in APC:Chl *a* (Fig. 1D). Between April and September the ratios were negatively correlated to the changes in daylength. PBtot:Chl *a* and APC:Chl *a* ratios were highest in June/July when the light was minimised to 5 h per day. Ratios began to decrease when daylengths increased. Between September and January, PBtot:Chl *a* ratios were constant whereas the APC:Chl *a* ratios showed a second small peak in November, gradually falling to their lowest levels in February.

The ratios of phycobiliproteins are shown in Fig. 1E. The PE:PC ratio and (slightly) the PE:APC ratio decreased from May onwards to their lowest values in October (with a slight peak in September), whereas the PC:APC ratio was more or less constant, although there may have been a slight increase in August/September. PE:APC and PE:PC increased again to their highest values in December/January. Even the PC:APC ratio was highest in January.

Lowest dry weights (DW) of 12.6–12.7% were found between April and June (Fig. 3). From July onwards, the DW increased continuously, with a slight peak in October, until February (up to 16.5% DW).

## Discussion

The results of this study demonstrate a strong relationship between the seasonal pattern of photosynthesis and pigments. The seasonal maximum of photosynthetic capacity in September/October is followed by a clear maximum in the concentrations of all pigments in October/November. This peak coincides in time with the natural sea-ice break-up in the Antarctic Peninsula region between early September and late November. Furthermore, the photosynthetic capacity increased in parallel with the phycobiliprotein content during the entire Antarctic autumn, winter and spring (from April/May to October). During this period, a positive correlation between total phycobiliprotein content and ETRmax was found, whereas a weaker correlation was found between Chl *a* and ETRmax. Weykam and Wiencke (1996) observed in a similar study with *P. decipiens* an optimum in oxygen production (per DW) 1 month later, in October/November, and maximal Chl *a* concentrations in December, with an increase during winter. The total phycobiliprotein content showed no clear seasonal pattern, even when related to FW. One reason

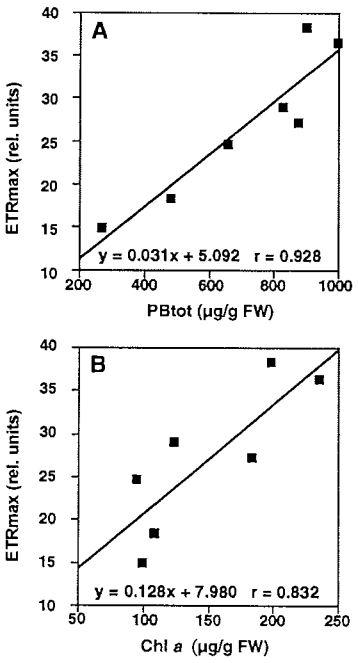


Fig. 2 Correlation of pigment contents and photosynthetic activity (*ETRmax*) between April/October: total pbycobiliprotein content (*PBtot*) and ETRmax (A) and Chl *a* content and ETRmax (B)

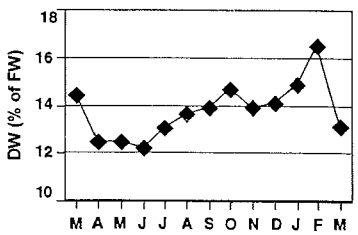


Fig. 3 Seasonal changes in dry weight (*DW*, % of fresh weight, *FW*). Means ± SD, $n = 10$. In those cases where no error bars are given, the SD is smaller than the symbol for the mean value

for differences in comparison with the present study may be the low number of replicates and the difference in age of the plants. All measurements were performed on only one old gametophyte, whereas in this study many 2-year young individuals were used. Nevertheless, changes in Chl *a* associated with changes in oxygen flux were also found in *P. decipiens* growing in different depths (Gómez et al. 1997). Similar seasonal photosynthetic patterns have been observed in several Antarctic brown macroalgae in the laboratory (Gómez and Wiencke 1997) and in the field (Gutkowski and Maleszewski 1989; Gómez et al. 1995, 1998). A clear seasonal variation in pigment pattern (Chl $a+c$), with a maximum in October coupled with an increase in photosynthetic activity, was found in *Adenocystis utricularis* (Gutkowski and Maleszewski 1989). Little seasonal variation of Chl *a*, with a peak in November, was observed in *Ascoseira mirabilis* (Gómez et al. 1995) and in *Desmarestia menziesii* (Gómez et al. 1998); however, a relationship between Chl *a* and thallus size rather than a relation between Chl *a* and light was emphasised by Gómez and Wiencke (1997).

Phycobiliprotein ratios may give further indications about changes in phycobilisome (PBS) number and size/structure. In general, PBS number and size (number of rods, rod length) can change according to acclimation processes, in response to light intensity, light quality or nutrient availability (Gantt 1990; Grossman et al. 1994; Talarico 1996). In autumn, the observed increase of APC content was due to an increase of PBS number. The decrease of PE:PC suggests a reduction of rod length and the slight increase of PC:APC suggests an increase of rod number. In summary, *P. decipiens* seems to acclimate during the autumn months by increasing the amount of "smaller" PBS (many rods with shorter rod length). In winter, the APC and PC contents were unchanged. But in September, the ratio of PC:APC was maximal and PE:PC, as well as PE:APC, increased slightly again, which suggests a slight increase in rod length. Thus, *P. decipiens* seems to acclimate during the winter months by slightly rising the PBS size (rod length). In early spring (October/November), the further increase of APC content to its highest value is due to a further increase in PBS number. From late spring (December) onwards the PBS amount seems to decline, but the high values of PE:PC and PE:APC suggest an increase in rod length of the remaining PBS. So in early spring, *P. decipiens* had the highest number of PBS, which it started to reduce in late spring, but the remaining PBS seems to be well assembled with prolonged rod length. In summer, the number of PBS further declined and the disruption of the remaining PBS started by reducing rod length, due to an decrease in PC:APC and PE:APC. These seasonal changes agree with observations in *Halopithys incurvus* (Rhodomelaceae) from the Mediterranean during an annual cycle; well-assembled PBS were found at low phycobiliprotein levels whereas small and incompletely assembled PBS occurred at high phycobiliprotein levels (Talarico and Kosovel 1983). In general, phycobiliprotein synthesis does not always lead to phycobilisome assembly (Lüning and Schmitz 1988; Foltran et al. 1996; Talarico 1996). So, the acclimation process of PBS discussed here should be proven by parallel electron microscopic studies.

The increase of phycobiliprotein content between April and June may be explained by photoacclimation to low light conditions, caused by decreasing daylength. This would result in maximal ratios of light-harvesting pigments to reaction centres and explains the maximum of PBtot:Chl *a* ratio and APC: Chl *a* ratio (Fig. 1D). The continuing increase of phycobiliprotein content from July on is presumably initiated by a specific seasonal trigger. Interestingly, it was also from July on that both the daily light dose and the Chl *a* content began to increase. The Chl *a* increase points to a rise in photosystem II reaction centres (RC II) and/or photosystem I reaction centres (RC I). The rise of Chl *a* may be coupled with an increase in thylakoid area, as shown in the red alga *Iridaea cordata* from Antarctica (Foltran et al. 1996). This species exhibits high levels of Chl *a*, combined with a thylakoid-rich chloroplast structure, when growing under the ice. In ice-free water, the chloroplasts possess a reduced thylakoid system and a lower Chl *a* content.

The increase in daylength may be a trigger for chlorophyll synthesis and a further production of phycobiliproteins. The hypothesis of a photoperiodic control of pigment synthesis in *P. decipiens* is possible, since different types of photoreceptors seem to control chlorophyll and phycobiliprotein synthesis in macroalgae (Rüdiger and López-Figueroa 1992; Häder and Figueroa 1997). López-Figueroa (1992) demonstrated that diurnal changes in the red/far-red (R:FR) ratio and in the blue:red ratio are well correlated with diurnal changes in photosynthetic pigment content in red macroalgae. Furthermore, annual variation of the end-of-day R:FR ratio is well correlated with the photoperiod, suggesting that it could act as an annual environmental light signal (Figueroa 1996). Annual changes in R:FR ratios have even been discussed as being responsible for modulating annual growth and reproduction in kelps (Lüning 1993; Häder and Figueroa 1997). The question is, do these wavelengths reach the algae growing in the sublittoral? The Antarctic water around King George Island was described as Jerlov water type III (Klöser et al. 1993), in which far-red is absorbed between 5 and 10 m depth and red light in 15 m depth. Therefore, the annual control by a red/far-red receptor is questionable; however, a control by a blue light receptor is possible. In this context, it is interesting that all phycobiliproteins show a common ultraviolet absorption band at 330 nm (Stadnichuk 1995), which may function as a photoreceptor. Furthermore, the chromophores of the phycobiliproteins are very similar to the phytochrome chromophore in higher plants, and have been suggested to control the chromatic adaptation in cyanobacteria (Grossman et al. 1994).

Another hypothesis is that the initiation of chlorophyll synthesis is triggered by growth. We know that

*P. decipiens* starts to grow in July, develops new blades during late winter/early spring (September) and exhibits optimal growth rates in October/November (Wiencke 1990b; Weykam and Wiencke 1996). This growth pattern is under photoperiodic control, and may have an underlying circannual rhythm, which may be triggered by daylength (Wiencke 1996). A good correlation between growth and daylength was also found in *D. menziesii* (Gómez and Wiencke 1997). Photoperiodic short-day responses are also responsible for the production of tetraspores, monospores, gametes, new blades and upright thalli in many algae, specially in red and brown macroalgae (Kain and Norton 1990; Lüning 1990).

We think that the extension of the daylength is a trigger to start growth and pigment synthesis in *P. decipiens*. The pigments increase number and/or size of light-harvesting antennae and reaction centres in order to trap as much light energy as possible, in order to optimise photosynthetic capacity and accelerate growth. This theory is supported by the fact that the pigment optimum reported in this study corresponds with the observed optimum in growth (Wiencke 1990b; Weykam and Wiencke 1996).

The continuously high Fv/Fm value of *P. decipiens* between May and December confirms that the photosynthetic apparatus remains intact during the entire mid-autumn, winter and spring. Conversely, the decrease of Fv/Fm, ETRmax and all pigments during summer suggests a degradation of the photosynthetic apparatus. The latter could be a normal photoacclimation process to high light, caused by the prolonged daylength, or also a photoinhibitory effect as demonstrated in *P. decipiens* in the field (Hanelt et al. 1994). A decrease of phycobiliproteins during summer was also found in *Gracilaria verrucosa* (Kosovel and Talarico 1979).

We conclude that *P. decipiens* is very well adapted to the short period of favourable light conditions in the field. *P. decipiens* maintains an intact photosynthetic apparatus during the entire mid-autumn, winter and spring. The alga starts to increase phycobiliprotein levels in mid-autumn, to build up new small phycobilisomes, the main light-harvesting antennae of red algae. Later, during winter, *P. decipiens* starts to increase Chl *a* levels to build up new reaction centres. This leads to a parallel increase of maximal photosynthetic activity. Subsequently, photosynthetic activity and pigment contents are maximal in Antarctic spring when the water is very clear and the sunlight penetrates deep into the water column, after break-up of sea ice and before the water becomes turbid due to plankton blooms and glacial melt-water. In contrast, during summer *P. decipiens* reduces its photosynthetic apparatus to a minimum: maximal quantum yield, maximal photosynthetic activity, and phycobiliprotein and Chl *a* levels are minimised. A photoperiodic control of pigment synthesis triggered by daylength is suggested. These results are in accordance with the life strategy of *P. decipiens* as season anticipator.

**Acknowledgements** This work was supported by grants from the Deutsche Forschungsgemeinschaft. We gratefully thank C. Langreder for excellent technical support, and K. Bischof and D. Hanelt for introducing the PAM technique. We acknowledge M. Schoenwaelder and I. Gómez for critically reading this manuscript. This is publication no. 1785 of the Alfred Wegener Institute.

## References

Bischof K, Hanelt D, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. Plant Biol 1:435–444

Figueroa FL (1996) Underwater light signals: role of algal photoreceptors in natural environment. G Bot Ital 130:608–623

Foltran A, Maranzana G, Rascio N, Scarabel L, Talarico L, Andreoli C (1996) *Iridaea cordata* from Antarctica: an ultrastructural, cytochemical and pigment study. Bot Mar 39:533–541

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of the red algae. Cambridge University Press, Cambridge, pp 203–219

Gómez I, Wiencke C (1997) Seasonal growth and photosynthetic performance of the Antarctic macroalga *Desmarestia menziesii* (Phaeophyceae) cultivated under fluctuating Antarctic daylengths. Bot Acta 110:25–31

Gómez I, Wiencke C, Weykam G (1995) Seasonal photosynthetic characteristics of *Ascoseira mirabilis* (Ascoseirales, Phaeophyceae) from King George Island, Antarctica. Mar Biol 123:167–172

Gómez I, Weykam G, Klöser H, Wiencke C (1997) Photosynthetic light requirements, metabolic carbon balance and zonation of sublittoral macroalgae from King George Island (Antarctica). Mar Ecol Prog Ser 148:281–293

Gómez I, Weykam G, Wiencke C (1998) Photosynthetic metabolism and major organic compounds in the marine brown alga *Desmarestia menziesii* from King George Island (Antarctica). Aquatic Bot 60:105–118

Grossman AR, Schaefer MR, Chiang GG, Collier JL (1994) The responses of cyanobacteria to environmental conditions: light and nutrients. In: Bryant DA (ed) The molecular biology of Cyanobacteria. Kluwer, Dordrecht, pp 641–675

Gutkowski R, Maleszewski S (1989) Seasonal changes of the photosynthetic capacity of the Antarctic macroalga *Adenocystis utricularis* (Bory) Skottsberg. Polar Biol 10:145–148

Häder DP, Figueroa FL (1997) Photoecophysiology of marine macroalgae. Photochem Photobiol 66:1–14

Hanelt D (1992) Photoinhibition of photosynthesis in marine macrophytes of the South Chinese Sea. Mar Ecol Prog Ser 82:199–206

Hanelt D, Huppertz K, Nultsch W (1992) Photoinhibition of photosynthesis and its recovery in red algae. Bot Acta 105:278–284

Hanelt D, Jaramillo J, Nultsch W, Senger S, Westermeier R (1994) Photoinhibition as a regulative mechanism of photosynthesis in marine algae of Antarctica. Ser Cient INACH 44:67–77

Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll *a* and *b* in *N,N*-dimethylformamide and 80% acetone. Plant Physiol 77:483–485

Kain JM, Norton TA (1990) Marine ecology. In: Cole KM, Sheath RG (eds) Biology of the red algae. Cambridge University Press, Cambridge, pp 377–422

Klöser H, Ferreyra G, Schloss I, Mercuri G, Laturnus F, Curtosi A (1993) Seasonal variation of algal growth conditions in sheltered Antarctic bays: the example of Potter Cove (King George Island, South Shetlands). J Mar Syst 4:289–301

Kosovel V, Talarico L (1979) Seasonal variations of photosynthetic pigments in *Gracilaria verrucosa* (Huds.) Papenfuss (Florideophyceae-Gigartinales). Boll Soc Adr Sci 63:5–15

López-Figueroa F (1992) Diurnal variation in pigment content in *Porphyra laciniata* and *Chondrus crispus* and its relation to the

diurnal changes of underwater light quality and quantity. Mar Ecol 13:285–305
Lüning K (1990) Seaweeds. Their environment, biogeography and ecophysiology. Wiley, New York
Lüning K (1993) Environmental and intertidal control of seasonal growth in seaweeds. Hydrobiologia 260:1–14
Lüning K, Schmitz K (1988) Dark growth of the red alga *Delesseria sanguinea* (Ceramiales): lack of chlorophyll, photosynthetic capability and phycobilisomes. Phycologia 27:72–77
Provasoli L (1966) Media and prospects for the cultivation of marine algae. Proc Jpn Conf Hakone:63–75
Rosenberg G (1981) Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp. (Chlorophyceae). PhD Thesis, Yale University
Rüdiger W, López-Figueroa F (1992) Photoreceptors in algae. Photochem Photobiol 55:949–954
Stadnichuk IN (1995) Phycobiliproteins: determination of chromophore composition and content. Phytochem Anal 6:281–288
Talarico L (1996) Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. Sci Mar 60:205–222
Talarico L, Kosovel V (1983) Ficobilisomi e variazioni delle ficobiliproteine in *Halopithys incurvus* (Huds.) Batt. (Rhodomelaceae-Rhodophyta) durante il ciclo annuale. Boll Soc Adr Sci 67:69–81
Weykam G, Wiencke C (1996) Seasonal photosynthetic performance of the endemic Antarctic alga *Palmaria decipiens* (Reinsch) Ricker. Polar Biol 16:357–361
Weykam G, Thomas DN, Wiencke C (1997) Growth and photosynthesis of the Antarctic red algae *Palmaria decipiens* (Palmariales) and *Iridaea cordata* (Gigartinales) during and following extended periods of darkness. Phycologia 36:395–405
Wiencke C (1990a) Seasonality of brown macroalgae from Antarctica – a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:589–600
Wiencke C (1990b) Seasonality of red and green macroalgae from Antarctica – a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601–607
Wiencke C (1996) Recent advances in the investigation of Antarctic macroalgae. Polar Biol 16:231–240

## *Publication 3*

Lüder UH, Knoetzel J and Wiencke C:

**New insights into phycobilisome structure and their variability according to seasonal changing light conditions in the Antarctic red macroalga *Palmaria decipiens***

(prepared to be submitted).

# New insights into phycobilisome structure and their variability according to seasonal changing light conditions in the Antarctic red macroalga *Palmaria decipiens*

Ulrike H. Lüder [1,2], Jürgen Knoetzel [2*], Christian Wiencke [1]

[1] Foundation Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

[2] Institute of Cell Biology, Biochemistry and Biotechnology, University of Bremen, Postbox 33 04 40, D-28334 Bremen, Germany

[*] present address
Novozymes A/S, Research and Development, Detergent Apllications, Krogshjvej 36, DK-2880 Bagsvaerd, Denmark

Author for correspondence:
Ulrike Lüder
ulueder@awi-bremerhaven.de

## ABSTRACT

The influence of seasonally fluctuating Antarctic daylength on the assembly of phycobilisomes was studied in *Palmaria decipiens* (Palmariales, Florideophyceae) in a long-term culture experiment, mimicking the Antarctic year. At monthly intervals, phycobilisomes were isolated and changes in phycobiliprotein contents and polypeptide composition were determined. Two phycobilisome forms, $PBS_{up}$ and $PBS_{low}$, and a seasonal variation of their number and size were detected. $PBS_{up}$ and $PBS_{low}$ acclimated to the seasonally changing light conditions by changing the PE:APC and PE:PC ratios, whereas the PC:APC remained constant. This indicates that the number of the rods remained unaltered, but the phycobilisome size was modulated by changing the PE part within the rods. The rod length seems to be altered primarily by coupling and uncoupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. The predominant $\gamma^{37.3}$-subunit varied only little during the entire year. A coupling of some $\gamma^{37.3}$-subunit is assumed in autum (August-June). In contrast, the $\gamma^{40.3}$-subunit does not vary with the seasons. The $\gamma^{33.5}$-subunit is located at the periphery of the rods, furthermore we propose a rod only consisting of $\gamma^{33.5}$-subunit and its associated PE hexamer. The predominant $\gamma^{37.3}$-subunit is located at the innermost PE part of the rod. The $\gamma^{40.3}$-subunit is suspected to be at a peripheral position, close to the thylakoid membrane.

The variation in the proportion of the two core membrane linkers $L_{CM}^{85.0}$ and $L_{CM}^{75.4}$ indicates an acclimation and/or regulation of the phycobilisome core.

'Small' phycobilisomes (lowest PE:APC and PE:PC ratios) were found in April (Antarctic summer/autumn). From April on a slight and from August (Antarctic winter) on a clear continuous increase in phycobilisome size until November was observed, resulting in 'large' phycobilisomes (highest PE:APC and PE:PC ratios) in November, both in $PBS_{up}$ and $PBS_{low}$. The relative proportion of $PBS_{up}$ and $PBS_{low}$ clearly changed during the Antarctic year. $PBS_{low}$ was nearly negligible in February. The appearance of $PBS_{up}$ and $PBS_{low}$ can be seen in relationship to the seasonal changes in the phycobiliprotein tissue content. A conversion of $PBS_{up}$ into $PBS_{low}$ is suggested. Different physiological functions to $PBS_{up}$ and $PBS_{low}$ are discussed. This is the first study showing the dynamics of phycobilisome assembly in response to seasonally fluctuating daylength.


## ABBREVIATIONS

APC, allophycocyanin; $L_C$ , core linker; $L_{CM}$ , core membrane linker; $L_R$, rod linker; $L_{RC}$, rod-core linker; PBS, phycobilisome; $PBS_{up}$ and $PBS_{low}$, upper and lower banded PBS,

isolated on discontinuous sucrose gradients in *P. decipiens*; PC, phycocyanin; PE, phycoerythrin.

## INTRODUCTION

Phycobilisomes are the main light-harvesting antennae in red algae. They are extra membraneous structures, attached to the stromal side of the thylakoid and consist of phycobiliproteins, which are connected by linker polypeptides (reviewed in Gantt 1990, Mörschel 1991, Reuter and Müller 1993, Sidler 1994, Bald et al. 1996, MacColl 1998, van Thor et al. 1998). The pigmented phycobiliproteins make up about 80 % and the linker polypepetides about 20 % of the phycobilisome molecular mass. Phycobilisomes are constructed of two main structural elements: a core in the centre and several peripheral cylindrical rods. In red algae, the common phycobiliproteins are phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). PE is located at the periphery of the rods, PC at the inner part of the rods and APC in the core. Phycobiliproteins are universally composed of α- and β-polypeptide subunits in equimolar (1:1) stoichiometry and with a molecular weight of 16-18 and 18-20 kDa, respectively, with one or more chromophores covalently attached (Stadnichuk 1995). They are organised *in vivo* in $(\alpha\beta)_3$ trimers and in $(\alpha\beta)_6$ hexamers, composed of two trimeric forms, which interact with specific linker polypeptides to form disc-shaped units, forming the core and the peripheral rod structures of a phycobilisome. Linker polypeptides are divided into four groups, according to their structural function in the phycobilisome (Glazer 1985, Reuter and Müller, 1993): the core linker ($L_C$) assembles the core structure; the core membrane linker ($L_{CM}$) attaches the phycobilisome to the thylakoid membrane and coordinates the assembly of APC trimers; the rod-core linker ($L_{RC}$) attaches the rods to the core; the rod linkers ($L_R$) are involved in the assembly of the peripheral rods. γ-subunits are coloured linkers with molecular masses of about 30 kDa and were originally found only in PE of red algae (Glazer and Hixon 1977). More recent studies have demonstrated their occurrence in PE of some marine cyanobacteria (Wilbanks and Glazer 1993, Swanson et al. 1991). 750 As linker polypeptides, they are located in the cavity of the hexameric PE structure. The γ-subunits of red algae carry four or five chromophores (Stadnichuk 1995). They are bifunctional, and act as light-harvesting phycobiliproteins and as rod linker polypeptides. In red algae, up to three different γ-subunits have been found (Stadnichuk 1995, Bernard et al. 1996, Stadnichuk et al. 1997, Ritz et al. 1998, Talarico et al. 1998).

The phycobilisome structure and the phycobiliprotein composition are highly variable in the course of acclimation to varying light intensities, light qualities and nutrient availability (Gantt 1990, Reuter and Müller 1993, Grossman et al. 1994, Talarico 1996). The phycobilisome number and/or the phycobilisome size are variable. The size can be modulated by reducing the rod length or even by the loss of hole rods. A modification of the core composition, changing the energy distribution is also possible. In cyanobacteria, even an exchange of phycobiliprotein classes occurred.

For this reason, seasonal changes in the amount and in the ratios of phycobiliproteins in *P. decipiens* (Lüder et al. 2001a), hypothesised changes in the phycobilisome number and size. Therefore, we were interested to the investigate the acclimation of phycobilisomes to changes in light conditions in more detail. In a previous study (Lüder et al. 2001b), we reported the unexpected finding of two hemiellipsoidal phycobilisome forms in *P. decipiens*. Two forms of phycobilisomes were also found in *Porphyridium purpureum*, and their variation in response to irradiance and nitrogen availability was demonstrated (Algarra and Rüdiger 1993). Two types (ellipsoidal and hemidiscoidal) of phycobilisomes have also been described in *Porphyra umbilicalis* from the field (Algarra et al. 1990), in *Porphyra yezoensis* during its life cycle (Shi et al. 1995), and in two species of cyanobacteria belonging to the *Phormidium* genus during chromatic adaptation (Westermann et al. 1993, Westermann and Wehrmeyer 1995). Moreover, we have identified in *P. decipiens* three coloured γ-subunits ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) and three associated PE-subunit complexes ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$) with different absorption characteristics around 544.5 nm and with a red shift in their absorption maxima. Furthermore, a red shift in their fluorescence emission maxima was detected, which probably improve the energy transfer downhill the rod. γ-subunits are indicators for coupling and uncoupling of PE within the phycobilisome. Thus, the loss of the terminal PE hexamers was accompanied by the loss of its associated γ-subunit, observed under high light conditions (Bernard et al. 1996). The level of transcripts encoding the γ-subunit change according to different environmental conditions (Apt et al. 1993). Furthermore, we have identified in *P. decipiens* an uncoloured 38.9 kDa polypeptide suspected to be the PC rod linker ($L_R^{38.9PC}$), an uncoloured 30.6 kDa polypeptide suspected to be the PC-APC rod-core linker ($L_{RC}^{30.6PC}$), and the small core linker ($L_C^{10}$), and the large anchor linker ($L_{CM}^{85.0}$) (Lüder et al. 2001b).

*P. decipiens* is endemic and one of the most common Antarctic red macroalgae living in the sublittoral. In the Antarctic, sublittoral algae are exposed to almost constant low water temperatures (-1.8 to +2.0 8C) and high nutrient concentrations over the entire year; therefore, the seasonal development of Antarctic macroalgae depends mainly on the strong

seasonal variation of light conditions, especially of the daylength (Wiencke 1996). Thus, we were able to monitor the seasonal development of Antarctic species in long-term culture experiments by changing the daylength weekly according to the seasonally fluctuating daylengths measured at the collecting site on King George Island, South Shetland Islands, Antarctica varying between 5 h in winter and 20 h in summer (Wiencke 1990a, 1990b; see also Fig. 1). *P. decipiens* has a life strategy as season anticipator. It is pseudoperennial and develops new blades during late winter/early spring (Wiencke 1990b). Growth starts in July and as is the case for photosynthesis, is maximal in spring (October/November) (Wiencke 1990b, Weykam and Wiencke 1996, Lüder et al. 2001a). A strong relationship between the seasonal pattern of pigments and of photosynthetic performance was found (Lüder et al. 2001a). Photosynthetic performance and pigment tissue content increases continuously in mid autumn and winter, reaching their highest values in spring. For this period of time a positive correlation between phycobiliprotein tissue content and photosynthetic performance and a weaker correlation between Chl *a* and photosynthetic performance was found. In summer, photosynthetic performance and all pigment levels decreased to their lowest values. (Lüder et al. 2001a).

In the present study, we describe the acclimation process of phycobilisome assembly to seasonally changing light conditions in a long-term culture study of *P. decipiens*. This is the first study, in which phycobilisome assembly was studied in an alga cultivated under seasonally fluctuating daylength (under constant low irradiance) over a time-scale of a year. The appearance of both phycobilisome forms was documented, and changes in the ratios of their phycobiliproteins as well as in their polypeptide composition were determined at monthly intervals. The results are discussed in relation to the phycobiliprotein tissue content and the photosynthetic performance (Lüder et al. 2001b).

## MATERIAL AND METHODS

### *Algal material and culture conditions*

Tetraspores of *Palmaria decipiens* (Reinsch) Ricker were collected on King George Island (South Shetland Islands, Antarctica, 62°12'S, 58°58'W) and the developing male gametophytes were cultivated in the laboratory under seasonally fluctuating daylengths (mimicking field conditions, Fig. 1) at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany (Wiencke 1988, Wiencke 1990a). Several young male gametophytes were grown in twenty-four 3 l glass beakers for about two years under seasonally fluctuating daylengths (Fig. 1), under a constant irradiance of 25 µmol

photons•$m^{-2}$•$s^{-1}$ using daylight fluorescent tubes (L58/W19, Osram, Munich, Germany) at a constant temperature of 0±18 °C in Provasoli enriched North Sea water (Provasoli 1966, 34 ‰, pH 8), aerated with pressurised air. To ensure sufficient nutrient supply, the culture medium was changed every second week.

In the first year a biomass of about 1200 g fresh weight was generated, and the alga was acclimated during this time to seasonally changing light conditions. At the beginning of the second year, in Antarctic March (14 h and 17 min light per day), the experiment was started and thalli were harvested from different beakers at monthly intervals and used for the isolation of phycobilisomes.

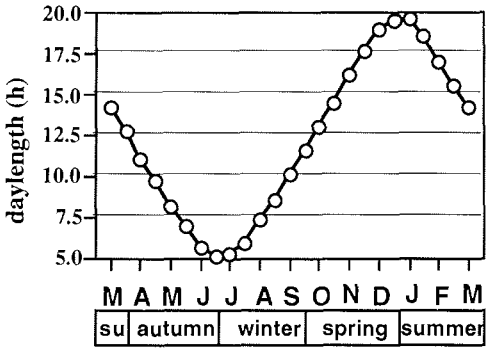


**Fig. 1.** Simulation of seasonal changes of daylength on King George Island, Antarctica. Summer/autumn change: 21. March, autumn/winter change: 21. June, winter/spring change: 21. September, spring/summer change: 21. December.

### *Isolation of phycobilisomes*

The phycobilisomes were isolated as previously described in Lüder et al. (2001b), according to Nies and Wehrmeyer (1980) and Reuter and Wehrmeyer (1990). Each month, approximately the same amount of fresh weight was disrupted by grinding and incubated in 1 % (w/v) N,N-dimethyl-dodecylamine-N-oxide (LDAO, Fluka, Buchs, Switzerland), deoxyribonuclease I (DNase I, 80 mg $ml^{-1}$ extract) and 15 % (w/v) sucrose in 1.5 M potassium phosphate buffer, pH 6.8, for 30 min in the dark at room temperature with gentle stirring. To prevent proteolytic degradation, all buffers contained 1 mM Pefabloc SC (Boehringer, Mannheim, Germany) and 2 mM EDTA. Cell debris was removed by centrifugation for 30 min at 48000 g (Sorvall RC-5B, SS34, Du Pont de Nemours, Bad Homburg, Germany). The supernatant was layered onto a step sucrose

gradient of 15, 25, 35 and 45 % (w/v) sucrose in 1.5 M potassium phosphate buffer, pH 6.8, and was ultracentrifuged at 280000 g and 13 °C for 22 h (L5-65, SW 40 Ti, Beckman, Fullerton, CA, USA). After centrifugation the phycobilisomes were collected with a syringe. The remaining phycobilisomes were pelleted by ultracentrifugation at 280000 g for 3 h (L5-65, Ti 50, Beckman), resuspended in 15 % (w/v) sucrose with 1 mM Pefabloc SC, frozen at -30 °C in aliquots, and used for subsequent electrophoresis.

### *SDS-PAGE*

The polypeptide composition of the phycobilisomes was analysed on 15 % (w/v) polyacrylamide gels as previously described in Lüder et al. (2001b), according to Reuter and Wehrmeyer (1988). For a better resolution of the linker polypeptides 6 % (v/v) isopropanol was added to the separation gel as described by Reuter and Nickel-Reuter (1993). Electrophoresis was performed on ice in a Mini Protean II cell (Bio-Rad) for 2h at 200 V. Samples containing 10 µg total protein each, were solubilized at 80 °C for 10 min in a buffer containing 5 % (w/v) SDS, 2 % (w/v) dithiothreitol, 20 % (w/v) sucrose, 0.25 M Tris HCl, pH 6.8 and 0.02 % (w/v) Coomassie Brillant Blue G250. After electrophoresis, gels were fixed for 1 h in 7 % (v/v) acetic acid with 40 % methanol, stained overnight in 0.025 % (w/v) Coomassie Brillant Blue R250 in 7 % (v/v) acetic acid and destained in 7 % (v/v) acetic acid.

The apparent molecular masses of polypeptides were calculated by using a broad range protein marker SDS-PAGE standard (Bio-Rad). The stained gels were scanned at 520-570 nm with a GS-700 Imaging Densitometer (Bio-Rad) with Multi-Analyst Software (Bio-Rad). The apparent molecular masses of all separated polypeptides were determined and their percentage distributions (% of total PBS protein, stainable with Coomassie blue) were calculated for each month.

### *Phycobiliprotein and protein determination*

The isolated phycobilisomes were dissociated in 0.1 M potassium phosphate buffer, pH 6.8, and the phycobiliprotein content in six different samples was determined by using the equations: PE (µg/ml) = 123.5 * $OD_{565}$ - 73.5 * $OD_{615}$ - 16.3 * $OD_{650}$, PC (µg/ml) = 163.2 * $OD_{615}$ - 117.1 * $OD_{650}$ and APC (µg/ml) = 165.6 * $OD_{650}$ - 16.4 * $OD_{615}$ after Rosenberg (1981).

The protein contents of the isolated PBS were determined with a Bio-Rad-Protein Assay (Bio-Rad, Munich, Germany), according to the instructions of the manufacturer.

## RESULTS

### *Intact phycobilisomes*

Intact undissociated phycobilisomes were isolated in the 35 % (w/v) sucrose layer as two discrete bands with seasonal variations in the appearance of the upper and lower banded phycobilisomes ($PBS_{up}$, $PBS_{low}$) (Fig. 2). Between February and May either $PBS_{up}$ or $PBS_{low}$ was present in high amounts, whereas the other one was found in very low amounts. In contrast, between June and January both $PBS_{up}$ and $PBS_{low}$ were present in high amounts, but with changing dominance.

In February, the amount of $PBS_{low}$ was almost negligible, recovered slightly in March and clearly increased in April and May. In contrast, between May and August, the amount of $PBS_{up}$ continuously increased, and suddenly decreased in September to a level, which it remained until March. Meanwhile, the amount of $PBS_{low}$ increased a second time in September and October, and decreased from November on; in February $PBS_{low}$ was almost negligible.

Dissociated PBS were found between the 15 % and 35 % (w/v) sucrose layers. The highest amount of dissociated PBS were observed between May and September. Free carotenoids and chlorophyll appeared in the 15 % (w/v) sucrose layer. A small dark green layer of membrane bound Chl and cell debris was found on the top of the gradients.

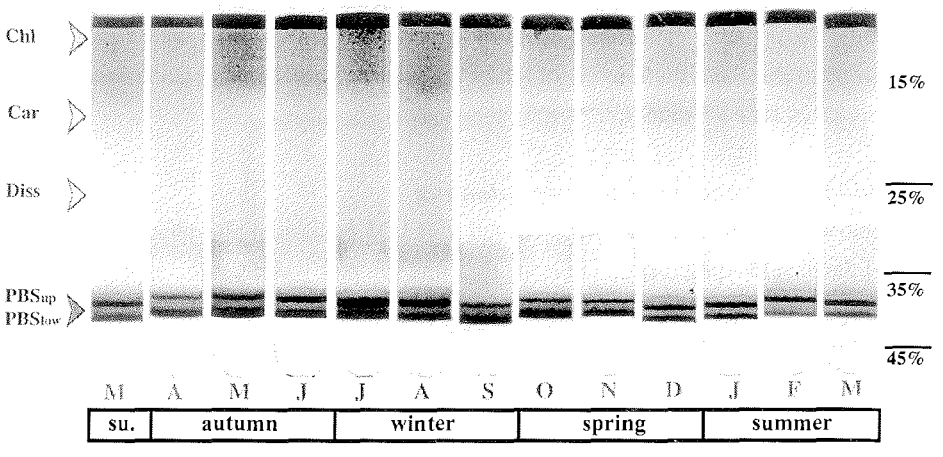


**Fig. 2.** Seasonal changes in the appearance of $PBS_{up}$ and $PBS_{low}$, isolated from *P. decipiens* in step sucrose density gradients of 15, 25, 35 and 45 % (w/v) sucrose. $PBS_{up}$ and $PBS_{low}$, intact phycobilisomes; diss, dissociated phycobilisomes; chl, chlorophyll; car, carotenoids.

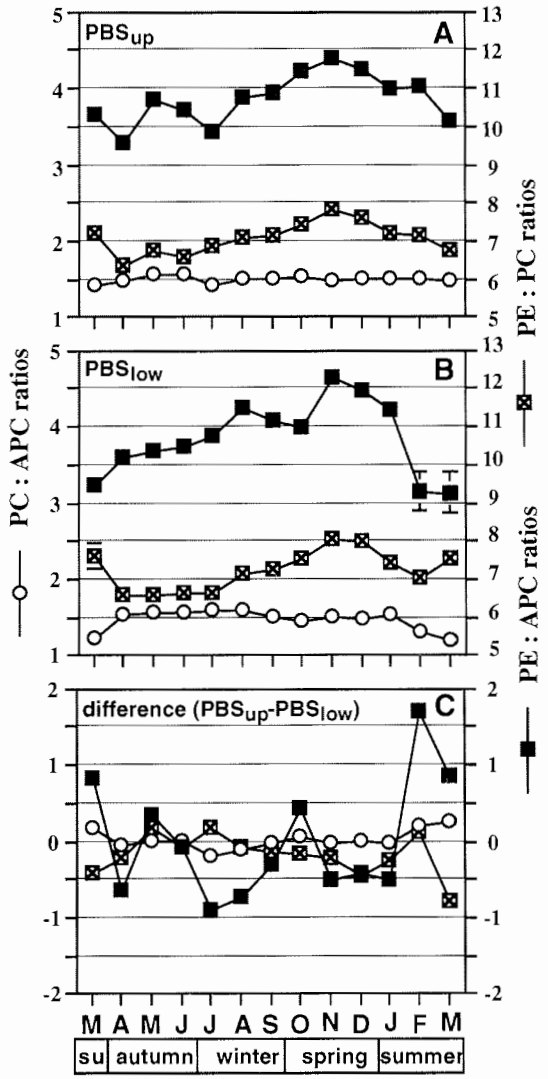


**Fig. 3.** Seasonal changes in the ratios of phycobiliproteins in the phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*. Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value. APC, allophycocyanin; PC, phycocyanin; PE, phycoerythrin.

***Phycobiliprotein ratios***

The seasonal changes of the PE:APC, PE:PC and PC:APC ratios of isolated phycobilisomes are shown in Fig. 3. $PBS_{up}$ and $PBS_{low}$ acclimated to the seasonally changing daylength by changing the PE:PC and PE:APC ratios, whereas the PC:APC ratio was nearly constant at 1.5, indicating no alteration in the number of rods. However, a slight decrease to 1.2-1.3 was noticed in $PBS_{low}$ in February and March, which would suggest a rod loss. The highest ratios of PE:APC and of PE:PC were reached in Antarctic spring (November) both in $PBS_{up}$ and $PBS_{low}$. The lowest ratios of PE:APC and of PE:PC were found just after Antarctic summer (April), both in $PBS_{up}$ and $PBS_{low}$. Thus, the phycobilisome size was smallest in April and largest in November, in the following called 'small' phycobilisomes and 'large' phycobilisomes.

During the entire year, the PE:APC ratio increased more or less continously slightly between April and July, both in $PBS_{up}$ and $PBS_{low}$. However, from August on, a clear continous increase in the PE:APC and PE:PC ratios until November was found. This points to a more or less continous increase in phycobilisome size from April until November, both in $PBS_{up}$ and $PBS_{low}$. From December on, when nearly the maximal daylength was reached, both $PBS_{up}$ and $PBS_{low}$ clearly started to decrease in size, continuing during the whole summer. In February, even a rod loss in $PBS_{low}$ is possible as indicated by the decrease of the PC:APC ratio.

In Fig. 3 C, the differences in the ratios of phycobiliproteins from $PBS_{up}$ and $PBS_{low}$ are represented, and no major differentiation in the phycobiliprotein composition and thus in the size between $PBS_{up}$ and $PBS_{low}$ is evident during the entire year.

***Linker polypeptides of the rods ($\gamma$-subunits, $L_R$ and $L_{RC}$)***

The seasonal changes of the linker polypeptides, described as percentage ratio of total PBS protein (% of total PBS protein, stainable with Coomassie blue), are presented in Fig. 4. The distribution of linker polypeptides belonging to the rod ($\gamma$-subunits, rod linker ($L_R$) and rod-core linker ($L_{RC}$)) from $PBS_{up}$ and $PBS_{low}$ is summarised in Fig. 4A and 4D. Both in $PBS_{up}$ and $PBS_{low}$, the $\gamma^{37.3}$-subunit has the highest protein part within all linker polypeptides and made up 4.9-5.7 % of total PBS protein, followed by the $\gamma^{40.3}$-subunit with 2.3-3.5 %, the $L_{RC}^{30.6PC}$ with 2.0-2.7 %, and the $L_R^{38.9PC}$ with about 1.4-2.2 %. The $\gamma^{33.5}$-subunit showed the greatest changes with about 1.5-2.9 % of total PBS protein during the entire year.

To exclude the influence of contaminants, and since the standard deviations of some samples were rather high, we calculated the percentage ratios (%-ratios) within the linker

polypeptides belonging to the rod (γ-subunits, $L_R$ and $L_{RC}$) (Fig. 5). First, a comparison of the 'small' and the 'large' phycobilisomes is made (see also Table 1). In the 'small' phycobilisomes (April), the %-ratio was maximal for $L_{RC}^{30.6PC}$, high for $L_R^{38.9PC}$, high or maximal for the $\gamma^{40.3}$-subunit, high for the $\gamma^{37.3}$-subunit and minimal for the $\gamma^{33.5}$-subunit. In contrast, in the 'large' phycobilisomes (November), the %-ratio was minimal for $L_{RC}^{30.6PC}$, low or minimal for $L_R^{38.9PC}$, low or minimal for the $\gamma^{40.3}$-subunit, middle or maximal for the $\gamma^{37.3}$-subunit and maximal for the $\gamma^{33.5}$-subunit. This suggest the location of the $\gamma^{40.3}$-subunit at the innermost, the $\gamma^{37.3}$-subunit in an intermediary position and the $\gamma^{33.5}$-subunit at the periphery of the PE part of the rods.

The seasonal variation of the linker polypeptides from $PBS_{up}$ and $PBS_{low}$ is very similar. In both $PBS_{up}$ and $PBS_{low}$, the $L_{RC}^{30.6PC}$ and the $L_R^{38.9PC}$ did not vary with the seasons, their %-ratios ranged only about 3%. In contrast, clear seasonal changes were seen in the $\gamma^{33.5}$-subunit. It showed in April with about 11 % the lowest ratio of the entire year and of all other linker polypeptides, but increased during autumn, winter and spring to its double ratio of about 20 % in November, both in $PBS_{up}$ and $PBS_{low}$. The opposite is seen for the $\gamma^{40.3}$-subunit. Its highest ratios are found in March/April (22 %/ 21 %), and the lowest in December/ November (19 %/ 17 %) in $PBS_{up}$ and $PBS_{low}$, respectively. However, this changes are so minimal, that we assume no alteration in the amount of the $\gamma^{40.3}$-subunit during the entire year. The lowest ratios of the predominant $\gamma^{37.3}$-subunit were seen in October (32 % and 33 %) both in $PBS_{up}$ and $PBS_{low}$. But the highest ratios were reached in June (38 %) for $PBS_{up}$ and in November (39 %) for $PBS_{low}$. The reason is, in $PBS_{up}$ and may be also in $PBS_{low}$, the amount of $\gamma^{37.3}$-subunit slightly increased in parallel with the $\gamma^{33.5}$-subunit until June. Then the amount of the $\gamma^{37.3}$-subunit remained unchanged but the amount of the $\gamma^{33.5}$-subunit continued to increase, which caused the decreased in the relative proportion of the $\gamma^{37.3}$-subunit.

***Linker polypeptides of the core ($L_{CM}$ and $L_C$)***

In Fig. 4B and 4E, the seasonal changes of the large core membrane linkers ($L_{CM}^{75.4}$ and $L_{CM}^{85.0}$), expressed as % of total PBS protein, are shown for $PBS_{up}$ and $PBS_{low}$. Between March and July, the $L_{CM}^{75.4}$ comprises about 1.5 % of total PBS protein and the $L_{CM}^{85.0}$ about 0.3 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$. Between September and January, however, $L_{CM}^{75.4}$ decreased to only 0.3 % and $L_{CM}^{85.0}$ reached 1.5% of total PBS protein. In February and March equal amounts of $L_{CM}^{75.4}$ and $L_{CM}^{85.0}$ were found in $PBS_{up}$ and $PBS_{low}$.

The seasonal changes in the small core Linker ($L_C$) and the 8.0 kDa polypeptide expressed as % of total PBS protein are shown in Fig. 4C and 4F for $PBS_{up}$ and $PBS_{low}$. $L_C^{10}$ made

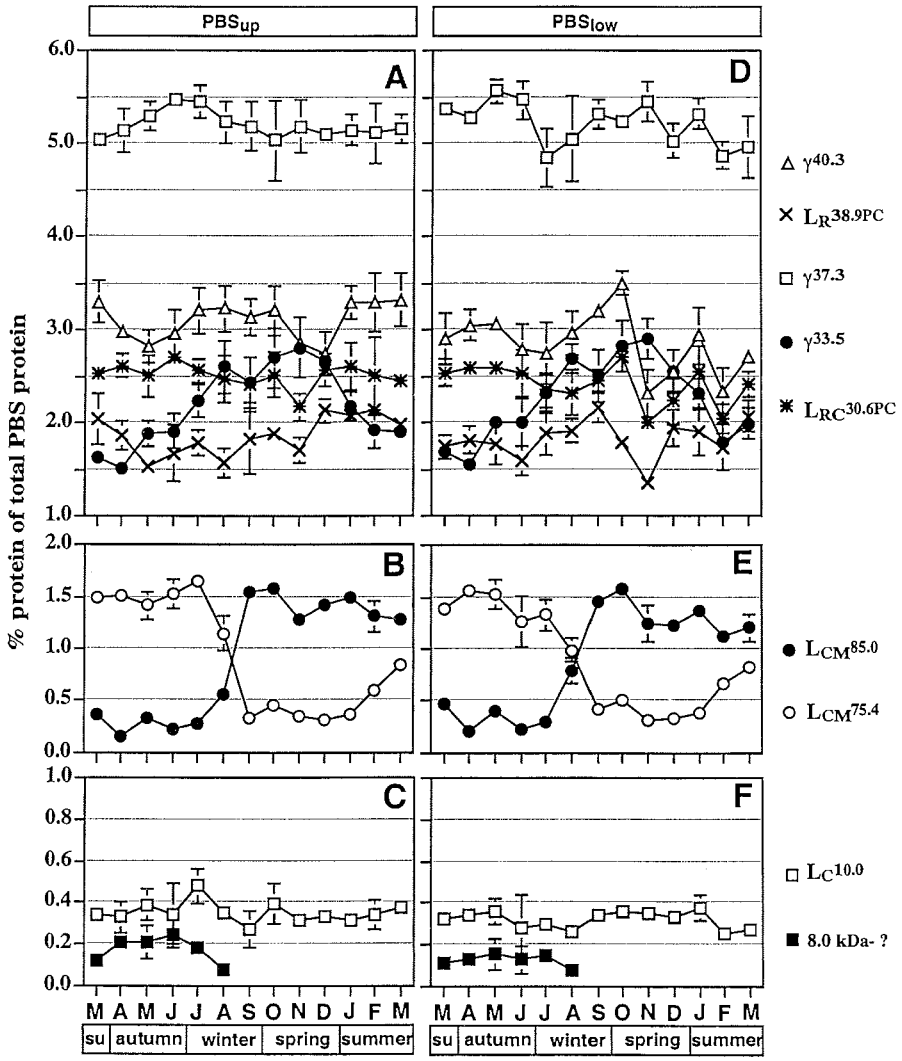


**Fig. 4.** Seasonal changes in the linker polypeptide composition of phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*. Represented as percentage ratio of total phycobilisome protein (% of total PBS protein), densitometrically calculated from the area under peaks of Coomassie blue stained SDS gel scans. Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value. $\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$, γ-subunits; $L_C$, core linker; $L_{CM}$, core membrane linker; $L_R^{PC}$, rod linker associated to phycocyanin;. $L_{RC}^{PC}$, rod-core linker associated to phycocyanin.

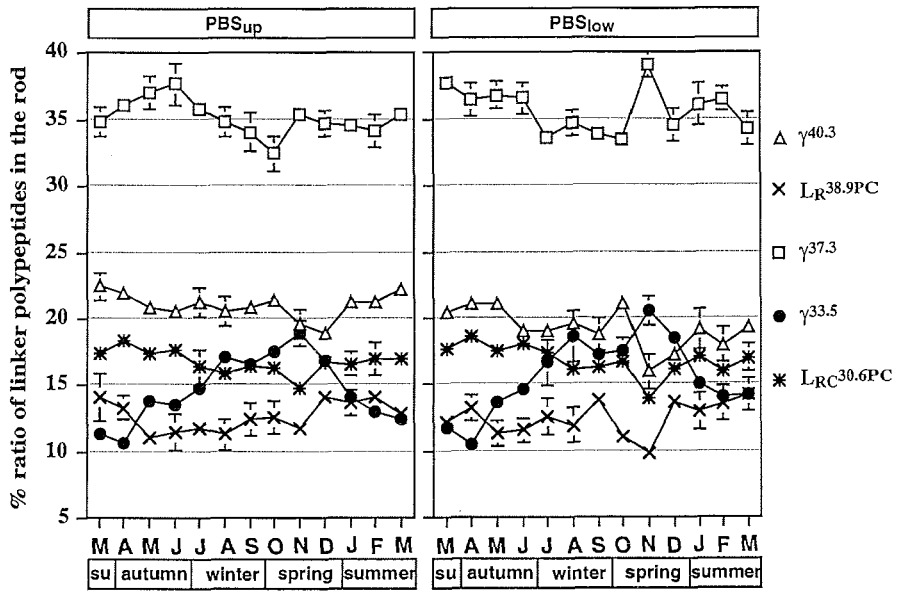


**Fig. 5.** Seasonal changes in the ratios of linker polypeptides belonging to the rods of phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*, and represented as percentage ratio (%-ratio). Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value. $\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$, γ-subunits; $L_R^{PC}$, rod linker associated to phycocyanin; $L_{RC}^{PC}$, rod-core linker associated to phycocyanin.

**Tab. 1.** Characterisation of the 'smallest', and 'largest' phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*, by the percentage ratios of their linker polypeptides belonging to the rods. Minimal (min), low, middle, high or maximal (max) occurrence in relation to the values found during the entire year (see Fig. 6); for $PBS_{low}$ the February and March values weren't taken into account.

| | $PBS_{up}$ | | | | $PBS_{low}$ | | | |
|---|---|---|---|---|---|---|---|---|
| linker | smallest PBS size (April) | | largest PBS size (November) | | smallest PBS size (April) | | largest PBS size (November) | |
| | % ratio | occurrence | % ratio | occurrence | % ratio | occurrence | % ratio | occurrence |
| $L_{RC}^{30.6}$ | 18.3 | max | 14.8 | min | 18.7 | max | 14.0 | min |
| $L_R^{38.9}$ | 13.3 | high | 11.8 | low | 13.3 | high | 9.9 | min |
| $\gamma^{40.3}$ | 21.9 | high | 19.6 | low | 21.1 | max | 16.0 | min |
| $\gamma^{37.3}$ | 36.1 | high | 35.4 | middle | 36.5 | high | 39.0 | max |
| $\gamma^{33.5}$ | 10.7 | min | 18.9 | max | 10.5 | min | 20.6 | max |

up about 0.3-0.4 % of total PBS protein, this amounts remained constant during the entire year, both in $PBS_{up}$ and $PBS_{low}$. An unknown 8.0 kDa polypeptide was found in the first part of the year, between March and August, and made up about 0.1-0.2 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$.

### *Contaminants*

The seasonal changes of the polypeptides thought to be contaminants (expressed as % of total PBS protein stainable with Coomassie) are summarised in Fig. 6.

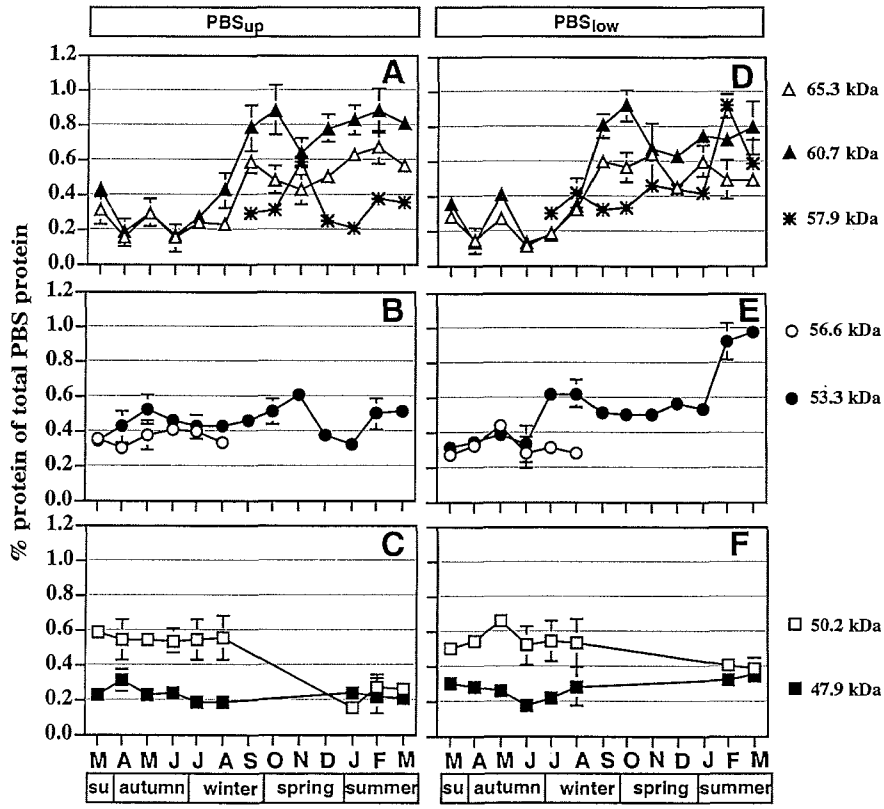


**Fig. 6.** Seasonal changes in the composition of polypeptides, thought to be contaminants or associated to the phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*. Represented as percentage ratio of total phycobilisome protein (% of total PBS protein), densitometrically calculated from the area under peaks of Coomassie blue stained SDS gel scans. Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value.

The 65.3 kDa, 60.7 kDa and 53.3 kDa polypeptides were present over the entire year. The 65.3 kDa and 60.7 kDa polypeptides were almost doubled in the second part of the year (from September on), both in $PBS_{up}$ and $PBS_{low}$. The 53.3 kDa polypeptide increased strongly in February and March in $PBS_{low}$, when compared with $PBS_{up}$.
The 56.6 kDa polypeptide was found only in the first part of the year (March-August), both in $PBS_{up}$ and $PBS_{low}$. In contrast, the 57.9 kDa polypeptide appeared only in the second part of the year (from September on). The 50.2 kDa and 47.9 kDa polypeptides were found between March and August, and again in January/February, both in $PBS_{up}$ and $PBS_{low}$.

## DISCUSSION

The main result of the present study is that the appearance of double banded phycobilisomes ($PBS_{up}$ and $PBS_{low}$) in *P. decipiens* varies over the entire year. The double banded phycobilisomes are not extraction artefacts or the result of partial proteolytic degradation (Lüder et al. 2001b). $PBS_{low}$ seems to be a closer packed and $PBS_{up}$ a more loosely aggregated hemiellipsoidal phycobilisome form. We suggest, that the differentiation between $PBS_{up}$ and $PBS_{low}$ is due to differences in aggregation rather than in phycobilisome size, since no differences in the phycobiliprotein ratios between $PBS_{up}$ and $PBS_{low}$ were found.

### *Seasonal changes in the phycobilisome size*

Both, $PBS_{up}$ and $PBS_{low}$ varied their size during the entire year. The phycobilisome size altered by changing the PE content, whereas the PC content remain constant. The invariance in the PC:APC ratios during the entire year, indicate no alterations in the number of rods. The seasonal changes in the PE:APC and PE:PC ratios indicate alterations of the rod length by coupling or uncoupling of PE. A variation of only PE was also described for other red algae during acclimation to different light conditions (Waaland et al. 1974, Kursar et al 1983, Chunningham et al. 1989, Algarra and Rüdiger 1993). In contrast, *Gracilaria verrucosa* acclimates to seasonal changes with changing the phycobilisome number, but without changing the phycobilisome size (constant ratios of PE:APC, PE:PC and PC:APC) (Kosovel and Talarico 1979). *Aglaothamnion neglectum* is another alga, which acclimates to irradiance only by changing the phycobilisome number and not the size (Apt and Grossman 1993).

The size of both $PBS_{up}$ and $PBS_{low}$ changed more or less in the same manner during the year. 'Smallest' phycobilisomes were found in April, both in $PBS_{up}$ and $PBS_{low}$. From April on and clearly from August on, the phycobilisome size increased more or less continously during autum, winter and spring to its 'largest' size in November, both in $PBS_{up}$ and $PBS_{low}$. From December on, when almost the maximal daylength was reached, $PBS_{up}$ and $PBS_{low}$ clearly started to decrease in size by continously uncoupling of PE during the hole summer. The prolonged daylength seems to lead to an excess of light energy (increase in daily light dosis), and may introduce the reduction in the phycobilisome size. Similarly, a decline in the PE:APC and/or PE:PC ratio was observed during acclimation to high irradiance (Waaland et al. 1974, Levy and Gantt 1988, Chunningham et al. 1989).

Another important point of this study is, that the present results do not correlate with the phycobiliprotein ratios obtained from phycobiliprotein tissue extracts in a previously performed study (Lüder et al. 2001a). This supports the strong need to investigate phycobilisomes and not only phycobiliproteins of tissue extracts of algae.

***Seasonal changes of rod linker polypeptides ($\gamma$, $L_R^{38.9PC}$, and $L_{RC}^{30.6PC}$)***

Alteration in the rod length was recognised in the seasonal changes of the percentage ratios (%-ratios) of the linker polypeptides belonging to the rods ($\gamma$-subunits, $L_R^{38.9PC}$ and $L_{RC}^{30.6PC}$). Both in $PBS_{up}$ and $PBS_{low}$, the $L_{RC}^{30.6PC}$ and the $L_R^{38.9PC}$ did not vary with the seasons. This agrees with the observed constant PC:APC ratios during the entire year.

Clear seasonal changes were seen only in the $\gamma^{33.5}$-subunit, whereas the $\gamma^{40.3}$-subunit did not vary, both in $PBS_{up}$ and $PBS_{low}$. The amount of the predominant $\gamma^{37.3}$-subunit also slightly increased, but only between April and June in $PBS_{up}$. Thus, the 'largest' phycobilisome size was achieved primarily by doubling the relative proportion of the $\gamma^{33.5}$-subunit, suggesting an elongation of the rods by an association of the $\gamma^{33.5}$-subunit and its associated PE hexamer. From December on the uncoupling of the $\gamma^{33.5}$-subunit started, probably caused by an excess of light energy, like discussed above. A loss of the terminal PE hexamers with its associated $\gamma$-subunit was also found under high light (Reuter and Müller 1993, Bernard et al. 1996).

An alteration of the $\gamma^{33.5}$-subunit and its associated PE hexamer also agrees with the finding of the $\gamma^{33.5}$-subunit in the trimeric PE subunit complex, suspected to be r-phycoerythrin (Lüder et al. 2001b). This r-phycoerythrin would be then the intermediary form during coupling/uncoupling of the hexameric form to/from the phycobilisome, as previously demonstrated with free PE (Algarra and Rüdiger 1993).

***The location of the γ-subunits within the rods***

The smallest $\gamma^{33.5}$-subunit and its associated PE hexamer is probably located at the periphery of the rods. A loss of the γ-subunit with the lowest molecular mass, and its location at the distal end of the rods, was also found in other red algae (Bernard et al. 1996, Apt et al. 2001). In contrast, Ritz et al. (1998) observed the loss of the γ-subunit with the highest molecular mass, in a mutant of *Porphyridium*, whereas the wild type showed all three γ-subunits. Furthermore, in this mutant, the rod-core linker (specific to PC) and phycocyanin were absent, and therefore a clear second PE fluorescence emission peak occurred at 605 nm (while only a shoulder was seen in the wild type). The authors suggested, that the remaining two γ-subunits of lower molecular masses and their associated PE hexamers transfer the excitation energy directly to APC. Consequently the wild type must contain rods, which only consist only of PE and transfer the energy with a high efficiency directly to APC. This may also be possible for *P. decipiens*, since the $\gamma^{33.5}$-subunit and its associated PE hexamer showed a fluorescence emission maximum at 582 nm and a second lower maximum at 595 nm. Furthermore, the low fluorescence observed at 618 nm and 632 nm (Lüder et al. 2001b) confirmed this. Thus, *P. decipiens* might modulate the phycobilisome size by alteration the length of rods, which are only or mainly consisting of PE hexamers associated with the $\gamma^{33.5}$-subunit. By this way a more efficient energy transfer, compared to the other hexameric PE subunit complexes is provided.

From its annual variation, the $\gamma^{37.3}$-subunit should be positioned at the intermediary position within the PE part of the rods. However, its associated PE hexamer shows maximal fluorescence with a slight red shift at 585 nm (Lüder et al. 2001b), which suggests clearly an innermost position.

From its annual variation, the $\gamma^{40.3}$-subunit should be positioned at the innermost PE part of the rod, but its associated PE hexamer shows maximal fluorescence at 581 nm (Lüder et al. 2001b), which suggests clearly a peripheral position. Since its associated PE hexamer appeared only in a minimal level, a special position is assumed, eg. close to the thylakoid membrane and at the periphery of the rods, may be attached to FNR (ferredoxin $NADP^+$ oxidoreductase).

Possible rod linker arrangements are summarised in Fig. 7 and a model of the largest phycobilisome from *P. decipens* is presented in Fig. 8.

***Seasonal changes of the linker polypeptides of the core ($L_{CM}$ and $L_C$)***

A very interesting feature are the seasonal changes of the two large core membrane linker $L_{CM}^{85.0}$ and $L_{CM}^{75.4}$. 'Small' phycobilisomes (in $PBS_{up}$ and $PBS_{low}$) were characterised by

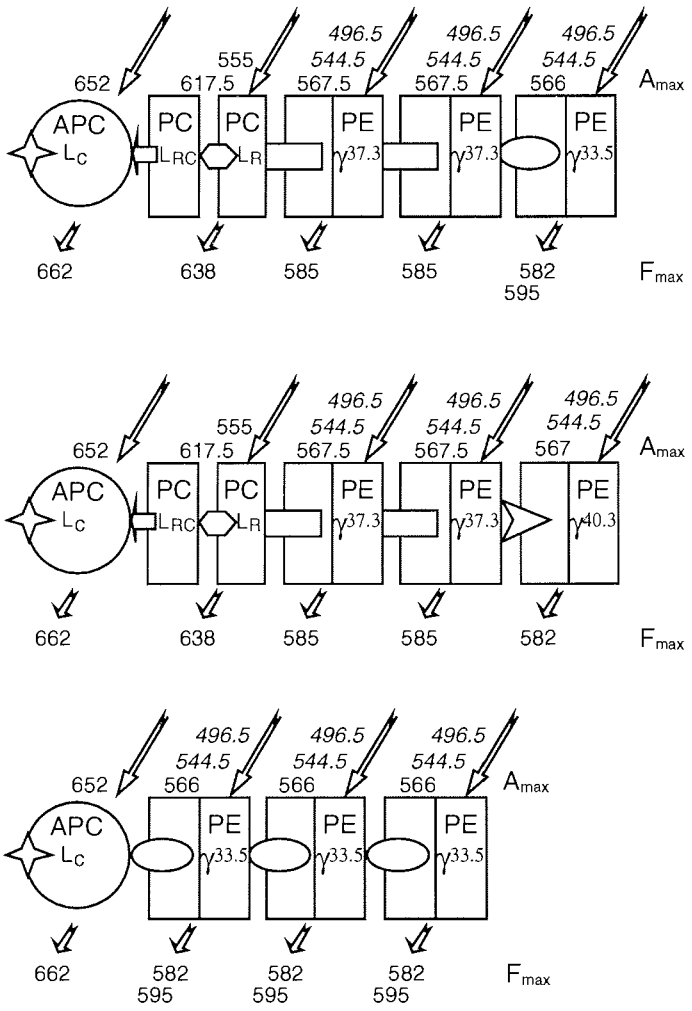


**Fig. 7.** Model of possible rod arrangements in *P. decipiens*. Allophycocyanin (APC), phycocyanin (PC) and phycoerythrin (PE) subunit complexes and their associated linkers are illustrated. Their absorption maxima ($A_{max}$) and fluorescence emission maxima ($F_{max}$) resulted from a previously study (Lüder et al. 2001b). $\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$, γ-subunits; $L_C$, core linker; $L_R^{PC}$, rod linker associated to phycocyanin;. $L_{RC}^{PC}$, rod-core linker associated to phycocyanin.

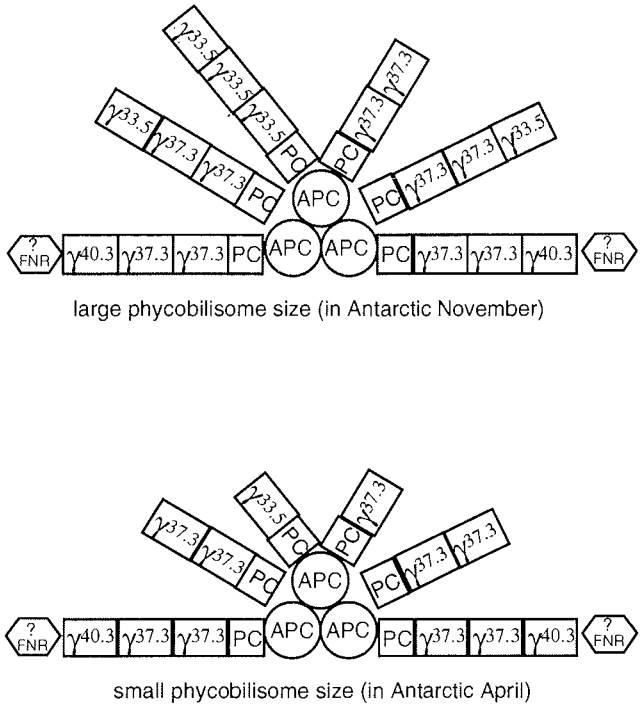


**Fig. 8.** Model of possible assembly of 'small' and 'large' phycobilisomes from in *P. decipiens*. Allophycocyanin (APC), phycocyanin (PC) and phycoerythrin subunit complexes are illustrated. $\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$, γ-subunits associated to phycoerythrin; FNR, Ferredoxin-NADP$^+$ Oxidoreductase. Since *P. decipiens* possess hemiellipsoidal phycobilisomes the double amount of rods should be assumed.

a higher abundance of the smaller $L_{CM}^{75.4}$, whereas the 'large' phycobilisomes were characterised by a higher abundance of the larger $L_{CM}^{85.0}$. This suggests an acclimation or regulation phenomena of the phycobilisome core. Two large polypeptides were often described in phycobilisome isolations and thought originally to be only dissociation products (Algarra and Rüdiger 1993). However in cyanobacteria it was demonstrated, that the molecular mass of $L_{CM}$ directly determines the core structure/size: a $L_{CM}^{72}$ formed a two cylinder core, a $L_{CM}^{94}$ and $L_{CM}^{99}$ a three cylinder core and a $L_{CM}^{128}$ resulted in a five cylinder core (Sidler 1994). Furthermore, per phycobilisome core two copies of $L_{CM}$ are

present, and reconstitution experiments showed that they also might be of different molecular masses (Esteban 1993, Gottschalk et al.1994).

Two different $L_{CM}$ ($L_{CM}^{120}$ and $L_{CM}^{105}$) were also found in 'small' phycobilisomes achieved under high light and low temperature, while only a single $L_{CM}$ ($L_{CM}^{120}$) occurred in 'large' phycobilisomes achieved under low light and high temperature in a cyanobacterium (Reuter and Nickel-Reuter 1993). Moreover, at high light irradiance or in red light an increase in an approximately 15 kDa smaller $L_{CM}$ was noticed, whereas its decrease at low light irradiance or in green light was observed. These observations are highly reproducible in cyanobacteria and in red algae; therefore an unspecified proteolytic degradation of the $L_{CM}$ is very unlikely and a post-translational modification of $L_{CM}$ was suggested (Reuter and Müller 1993). Modifications of the core composition seem also to be involved in acclimation processes. This is a new aspect, since over a long period of research on phycobilisomes, it has been postulated that the core composition is constant at all culture conditions (Reuter and Müller 1993).

The second linker type involved in the assembly of the core, the small core linker $L_C^{10}$, was present more or less in the same abundance over the entire year. A second low molecular mass polypeptide of 8.0 kDa present only in the first part of the year might be a second core linker, like previously discovered in *Rhodella violacea* (Reuter et al. 1990).

***Seasonal changes of the linker polypeptides thought to be contaminants***

The remaining polypeptides were often found in SDS gels of phycobilisomes. A 47 kDa polypeptide was identified as FNR (ferredoxin $NADP^+$ oxidoreductase), which is associated with the phycobilisomes, probably attached to the peripheral rods (Sidler 1994, Ritz et al.1998). Proteolytic enzymes of 50-66 kDa, which are involved in the degradation of the linker polypeptides, were found even in highly purified phycobiliprotein complexes of phycobilisomes (Reuter and Müller 1993). A 54 kDa polypeptide was identified as the large subunit of Rubisco and was interpreted to be a contaminant (Marquardt et al. 1999). Thus, the 60.7 kDa and 65.3 kDa polypeptides, which increased clearly from September on, might be proteases responsible for the coupling/uncoupling of PE.

***Seasonal variation in the appearance of $PBS_{up}$ and $PBS_{low}$***

The variation in the appearance of $PBS_{up}$ and $PBS_{low}$ during the Antarctic year seems to be coupled with the seasonal changes of daylength and with the seasonal changes of the phycobiliprotein tissue content (Lüder et al. 2001a). Lowest phycobiliprotein tissue content (calculated per fresh weight) was found in summer, between February and April. From May onwards, all phycobiliproteins (APC, PC and PE) increased more or less

continuously to their highest values in October (APC and PC) and in November (PE). A marked decrease in the APC and PC tissue contents were noticed in December, while PE remained unchanged. An almost halving of the tissue content of PE, APC and PC was noticed in February.

However, during times of lower phycobiliprotein tissue contents, only one of the two phycobilisome forms was present in high amounts, while the other one almost disappeared (between February and May). During the times of higher phycobiliprotein tissue contents, both PBS forms were present at high amounts, but with changing dominance (June-January).

The decreasing daylength in February (summer) seems to induce the loss of $PBS_{low}$, resulting in a halving of APC, PC and PE tissue contents to their minimal values, and moreover in lowest APC:Chl *a* and total phycobiliprotein:Chl *a* proportions of the entire year. After the 12 h daylength in April, there appears to be a conversion of $PBS_{up}$ into $PBS_{low}$, since the phycobiliprotein tissue content was still considerably low, and especially the APC tissue content was unchanged, but the amount of $PBS_{up}$ decreased and the amount of $PBS_{low}$ increased. In May, when the phycobiliprotein tissue content started to increase, the amount of $PBS_{low}$ increased further. In June and July, the amount of $PBS_{up}$ clearly increased. Thus, *P. decipiens* seems to acclimate to the shortest daylength by increasing the amount of $PBS_{up}$, resulting in the highest APC:Chl *a* and total phycobiliprotein:Chl *a* proportion of the entire year (Lüder et al. 2001a). In August and September, the APC and PC tissue contents were unchanged, but in September the amount of $PBS_{up}$ decreased to a moderate level for the rest of the year. The amount of $PBS_{low}$ increased, suggesting a conversion of $PBS_{up}$ to $PBS_{low}$. In October, the APC and PC tissue contents increase to their highest values, and $PBS_{low}$ was still much more dominant than $PBS_{up}$. From December onwards, APC and PC content, and the amount of $PBS_{low}$ decreased in a manner, that $PBS_{up}$ and $PBS_{low}$ appeared in nearly equal amounts in December and January. In February, $PBS_{low}$ was almost negligible.

Two phycobilisome populations with different aggregation states ('soluble' and 'aggregated' PBS) and their variation in appearance according to light irradiance and nitrogen avaiblability could also been shown in *Porphyridium purpureum* (Algarra and Rüdiger). The acclimation to low light occurred by increasing the amount and the size of 'soluble' PBS, with parallel decreasing the amount of 'aggregated' PBS. Acclimation to high light occurred by decreasing the amount and the size of the 'soluble' PBS, with parallel increasing of the amount of 'aggregated' PBS, which also decreased in size. However, at the end of the acclimation processes, 77% of total PE tissue content was associated to the 'soluble' PBS form and only 12% to the 'aggregated' PBS form at low

light, and the opposite was achieved at high light. This results show, that a conversion of both phycobilisome populations is possible and would support the theory of a conversion of $PBS_{up}$ into $PBS_{low}$ in *P. decipiens*. Algarra and Rüdiger (1993) interpreted, that only the 'soluble' PBS appears to be able to photoacclimate, but not 'aggregated' PBS. The 'aggregated' PBS was predominant under high light and nitrogen limitation.

There is a strong relationship between the seasonal changes of phycobiliprotein tissue content and of photosynthetic performance (Lüder et al. 2001a). The variation in the appearance of $PBS_{up}$ and $PBS_{low}$ during the Antarctic year seems to be related to the seasonal changes in optimal quantum yield and photosynthetic performance. However, in summer (February) the loss of $PBS_{low}$ was accompanied by a marked fall in the Chl *a* tissue content and a clear reduction in optimal quantum yield and photosynthetic performance. In contrast, the conversion from $PBS_{up}$ to $PBS_{low}$ in April was accompanied by an increase in optimal quantum yield, and the increase in the amount of $PBS_{low}$ in May was accompanied by an increase in photosynthetic performance, while the Chl *a* tissue content remained unchanged until June. Furthermore, the conversion from $PBS_{up}$ to $PBS_{low}$ in September, was accompanied by a sudden increase in photosynthetic performance. We suppose, that $PBS_{low}$ stimulates or enhances the photosynthetic performance. In contrast, $PBS_{up}$ appears to be the basic light harvesting antennae or even a pre-phycobilisome to build up $PBS_{low}$, when an enhancement in photosynthetic performance is required.

***Conclusion***

The phycobilisomes of *P. decipiens* possess a broad range of variability. The number and the size of $PBS_{up}$ and $PBS_{low}$ is variable. A conversion of $PBS_{up}$ into $PBS_{low}$ is possible. The phycobilisome size is modulated primary by the coupling and uncoupling of the smallest $\gamma^{33.5}$-subunit and its associated PE hexamer. This PE subunit complex has a further fluorescence emission maximum at 595 nm, which enhances the energy transfer downhill the rods. Furthermore, rods only or mainly consisting of this PE complex are possible. Moreover, an acclimation of the phycobilisome core was shown. An alteration of the PBS:(PSII and/or PS I) stoichiometry happened.

From April onwards the number of $PBS_{up}$ and/or $PBS_{low}$ and the size of $PBS_{up}$ and $PBS_{low}$ increases more or less continuously until November. From December on, the number of $PBS_{low}$ and the size of $PBS_{up}$ and $PBS_{low}$ decreases more or less continuously during the summer months.

*P. decipiens* acclimates to the shortest daylength mainly by increasing the amount of $PBS_{up}$, but also by a slight increase in the size of $PBS_{up}$ and $PBS_{low}$. *P. decipiens* acclimates to the longest daylength and in the summer months mainly by decreasing the amount of $PBS_{low}$, but also by decrease in the size of $PBS_{up}$ and $PBS_{low}$.

The presence of $PBS_{low}$ seems to enhance the photosynthetic performance. We think, *P. decipiens* enhances photosynthetic performance initially in April (early Antartic autumn) by conversion of the existing $PBS_{up}$ into $PBS_{low}$ and increasing the amount of $PBS_{low}$. In June, July and August (Antarctic autumn/winter), $PBS_{up}$ is the main phycobilisome form. In September $PBS_{up}$ is conversed into $PBS_{low}$, which enhances photosynthetic performance to its maximal values in September/October. This is the time, when in Antarctica the sea ice breaks up, and when *P. decipiens* has to produce biomass and reserves in a short light window (Wiencke 1990b, Weykam and Wiencke 1996). In December, with the decrease in the amount of $PBS_{low}$ the photosynthetic performance started to decrease.

## ACKNOWLEDGEMENTS

This work was supported by grants from the Deutsche Forschungsgemeinschaft. We gratefully thank Erika Lorenz and Ute Helmboldt for excellent technical support We acknowledge J. Marquardt for helpful comments.

## REFERENCES

Algarra P, Niell FX (1990) Short-term pigment response of *Corallina elongata* Ellis et Solander to light intensity. Aquat Bot 36:127-138

Algarra P, Rüdiger W (1993) Acclimation processes in the light harvesting complex of the red alga *Porphyridium purpureum* (Bory) Drew et Ross, according to irradiance and nutrient availability. Plant Cell Environ 16:149-159

Apt KE, Grossman AR (1993) Characterization and transcript analysis of the major phycobiliprotein subunit genes from *Aglaothamnion neglectum* (Rhodophyta). Plant Mol Biol 21:27-38

Apt KE, Hoffman NE, Grossman AR (1993) The γ subunit of R-phycoerythrin and its possible mode of transport into the plastid of red algae. J Biol Chem 268:16208-16215

Apt KE, Metzner S, Grossman AR (2001) The γ subunits of phycoerythrin from red alga: position in phycobilisomes and sequence characterization. J Phycol 37:64-70

Bald D, Kruip J, Rögner M (1996) Supramolecular architecture of cyanobacterial thylakoid membranes: How is the phycobilisome connected with the photosystems ? Photosynth Res 49:103-118

Bernard C, Etienne A-L, Thomas J-C (1996) Synthesis and binding of phycoerythrin and its associated linkers to the phycobilisome in *Rhodella violacea* (Rhodophyta):compared effects of high light and translation inhibitors. J Phycol 32:265-271

Cunningham FX Jr, Dennenberg RJ, Mustardy L, Jursinic PA, Gantt E (1989) Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga *Porphyridium cruentum* as a function of growth irradiance. Plant Physiol 91:1179-1187

Esteban MA (1993) Sequenzierung, Klonierung und Characterisierung der Gene von Core-Polypeptiden aus dem Cyanobacterium *Mastigocladus laminosus*. PhD thesis No 10040, Eidgenössische Technische Hochschule, Zürich, Switzerland

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of the Red Algae. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney, pp 203-219

Glazer AN (1985) Light harvesting by Phycobilisomes. Annu Rev Biophys Biophys Chem 14:47-77

Glazer AN, Hixson CS (1977) Subunit structure and chromophore composition of rhodophytan phycoerythrins *Porphyridium cruentum* B-phycoerythrin and b-phycoerythrin. J Biol Chem 252:32-42

Gottschalk L, Lottspeich F, Scheer H (1994) Reconstitution of allophycocyanin from *Mastigocladus laminosus* with isolated linker polypeptides. Photoche Photobiol 58:761-767

Grossman AR, Schaefer MR, Chiang GG, Colier JL (1994) The responses of cyanobacteria to environmental conditions: light and nutrients. In: Bryant DA (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Netherlands, pp 641-675

Kosovel V, Talarico L (1979) Seasonal variations of photosynthetic pigments in *Gracilaria verrucosa* (Huds) Papenfuss (Florideophyceae-Gigartinales). Boll Soc Adr Sci 63:5-15

Kursar TA, van der Meer J, Alberte RS (1983) Light-harvesting system of the red alga *Gracilaria tikvahiae*. II. Phycobilisome characteristics of pigment mutants. Plant Physiol 73:361-369

Levy I, Gantt E (1988) Light acclimation in *Porphyridium purpureum* (Rhodophyta):Growth, photosynthesis, and phycobilisomes. J Phycol 24:452-458

Lüder UH, Knoetzel J, Wiencke C (2001a) Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. Polar Biol 24:598-603

Lüder UH, Knoetzel J, Wiencke C (2001b) Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae). Physiol Plant 112:572-581

MacColl R (1998) Cyanobacterial phycobilisomes. J Struct Biol 124:311-334

Marquardt J, Schultze A, Rosenkranz V, Wehrmeyer W (1999) Ultrastructure and photosynthetic apparatus of *Rhodella violacea* (Porphyridiales, Rhodophyta) grown under iron-deficient conditions. Phycologia 38:418-427

Mörschel E (1991) The light-harvesting antennae of cyanobacteria and red algae. Photosynthetica 25:137-144

Nies M, Wehrmeyer W (1980) Isolation and biliprotein characterization of phycobilisomes from the thermophilic Cyanobacterium *Mastigocladus laminosus*. Planta 150:330-337

Provasoli L (1966) Media and prospects for the cultivation of marine algae. Proc Jpn Conf Hakone 63-75

Reuter W, Müller C (1993) Adaptation of the photosynthetic apparatus of cyanobacteria to light and $CO_2$. J Photochem Photobiol B:Biol 21:3-27

Reuter W, Nickel C, Wehrmeyer W (1990) Isolation of allophycocyanin B from *Rhodella violacea* results in a model of the core from hemidiscoidal phycobilisomes of Rhodophyceae. FEBS Lett 273:155-158

Reuter W, Nickel-Reuter C (1993) Molecular assembly of the phycobilisomes from the cyanobacterium *Mastigocladus laminosus*. J Photochem Photobiol B:Biol 18:51-66

Reuter W, Wehrmeyer W (1988) Core substructure in *Mastigocladus laminosus* phycobilisomes. I. Microheterogeneity in two of three allophycocyanin core complexes. Arch Microbiol 150:534-540

Reuter W, Wehrmeyer W (1990) Core substructure in *Mastigocladus laminosus* phycobilisomes. II. The central part of the tricylindrical core - $AP_{CM}$ - contains the "anchor" polypeptide and no allophycocyanin B. Arch Microbiol 153:111-117

Ritz M, Lichtlé C, Spilar A, Joder A, Thomas J-C, Etienne A-L (1998) Characterization of phycocyanin-deficient phycobilisomes from a pigment mutant of *Porphyridium* sp. (Rhodophyta). J Phycol 34:835-843

Rosenberg G (1981) Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp (Chlorophyceae). Ph D thesis, Yale University, New Haven, Connecticut

Shi D-J, Peng G-H, Chen JX, Wang H-J, Jin P, Feng LJ, Zhang H, Fei XG, Tseng CK, Tang PS (1995) Possible occurrence and characteristics of two types of phycobilisomes during ontogenesis of red alga *Porphyra yezoensis*. In: Mathis P (ed) Photosynthesis: from Light to Biosphere, Vol I. Kluwer Academic Publishers, Netherlands, pp 383-386

Sidler WA (1994) Phycobilisome and phycobiliprotein structures In: Bryant, D A (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Netherlands, pp 139-216

Stadnichuk IN (1995) Phycobiliproteins: determination of chromophore composition and content. Phytochemical Analysis 6:281-288

Stadnichuk IN, Karapetyan NV, Kislov LD, Semenenko VE, Veryasov MB (1997) Two γ-polypeptides of B-phycoerythrin from *Porphyridium cruentum*. J Photochem Photobiol B:Biol 39:19-23

Swanson RV, Ong LJ, Wilbanks SM, Glazer AN (1991) Phycoerythrins of marine unicellular cyanobacteria. II. Characterization of phycobiliproteins with unusually high phycourobilin content. J Biol Chem 266:9528-9534

Talarico L (1996) Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. Sci Mar 60:205-222

Talarico L, Rascio N, Dalla Vecchia F, Maranzana G (1998) Some observations on phycobilisomes of *Pterocladiella capillacea* (Gelidiaceae, Rhodophyta). Plant Biosystems 132:87-96

van Thor J J, Mullineaux CW, Matthijs HCP, Hellingwerf KJ (1998) Light harvesting and state transitions in cyanobacteria. Bot Acta 111:430-443

Waaland JR, Waaland SD, Bates G (1974) Chloroplast structure and pigment composition in the red alga *Griffithsia pacifica*: regulation by light intensity. J Phycol 10:193-199

Westermann M, Reuter W, Schimek C, Wehrmeyer W (1993) Presence of both hemidiscoidal and hemiellipsoidal phycobilisomes in a *Phormidium* species (Cyanobacteria). Z Naturforsch Teil C: Biochem Biophys Biol Virol 48:28-34

Westermann M, Wehrmeyer W (1995) A new type of complementary chromatic adaptation exemplified by *Phormidium* sp. C86: changes in the number of peripheral rods and in the stoichiometry of core complexes in phycobilisomes. Arch Microbiol 164:132-141

Weykam G, Wiencke C (1996) Seasonal photosynthetic performance of the endemic Antarctic alga *Palmaria decipiens* (Reinsch) Ricker. Polar Biol 16:357-361

Wiencke C (1988) Notes on the development of some benthic marine macroalgae of King George Island, Antarctica. Ser Cient INACH 37:23-47

Wiencke C (1990a) Seasonality of brown macroalgae from Antarctica - a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:589-600

Wiencke C (1990b) Seasonality of red and green macroalgae from Antarctica - a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601-607

Wiencke C (1996) Recent advanes in the investigation of Antarctic macroalgae. Polar Biol 16:231-240

Wilbanks SM, Glazer AN (1993) Rod structure of a phycoerythrin II-containing phycobilisome. II. Complete sequence and bilin attachment site of a phycoerythrin γ-subunit. J Biol Chem 268:1236-1241

*Publication 4*

Lüder UH, Wiencke C and Knoetzel J :

# Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta) - a study to simulate Antarctic winter sea ice cover.

Journal of Phycology (2002) 38: 904-913

*J. Phycol.* 38, 904–913 (2002)

# ACCLIMATION OF PHOTOSYNTHESIS AND PIGMENTS DURING AND AFTER SIX MONTHS OF DARKNESS IN *PALMARIA DECIPIENS* (RHODOPHYTA): A STUDY TO SIMULATE ANTARCTIC WINTER SEA ICE COVER[1]

*Ulrike H. Lüder,*[2] *Christian Wiencke*

Foundation Alfred Wegener Institute, Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

*and*

*Jürgen Knoetzel*[3]

Institute of Cell Biology, Biochemistry and Biotechnology, University of Bremen, Postbox 330440, D-28334 Bremen, Germany

The influence of seasonally fluctuating photoperiods on the photosynthetic apparatus of *Palmaria decipiens* (Reinsch) Ricker was studied in a year-round culture experiment. The optimal quantum yield ($F_v/F_m$) and the maximal relative electron transport rate ($ETR_{max}$), measured by *in vivo* chl fluorescence and pigment content, were determined monthly. During darkness, an initial increase in pigment content was observed. After 3 months in darkness, $ETR_{max}$ and $F_v/F_m$ started to decrease considerably. After 4 months in darkness, degradation of the light-harvesting antennae, the phycobilisomes, began, and 1 month later the light harvesting complex I and/or the reaction centers of PSII and/or PSI degraded. Pigment content and photosynthetic performance were at their minimum at the end of the 6-month dark period. Within 24 h after re-illumination, *P. decipiens* started to accumulate chl *a* and to photosynthesize. The phycobiliprotein accumulation began after a time lag of about 7 days. *Palmaria decipiens* reached $ETR_{max}$ values comparable with the values before darkness 7 days after re-illumination and maximal values after 30 days of re-illumination. Over the summer, *P. decipiens* reduced its photosynthetic performance and pigment content, probably to avoid photodamage caused by excess light energy. The data show that *P. decipiens* is able to adapt to the short period of favorable light conditions and to the darkness experienced in the field.

*Key index words:* Antarctica; darkness; day length; *Palmaria decipiens*; photoacclimation; photosynthesis; phycobiliproteins; Rhodophyta; seasonality

*Abbreviations:* APC, allophycocyanin; DW, dry weight; ETR, relative electron transport rate; $ETR_{max}$, maximal ETR; $F_0$, $F_m$, and $F_v$, minimal, maximal, and variable, respectively, chl fluorescence of PSII after dark incubation; $F_v/F_m$, optimal quantum yield of PSII in dark-acclimated state; $F_0'$, $F_m'$, and $F_s$, minimal, maximal, and steady-state, respectively, chl fluorescence of PSII after light incubation; $\Delta F$, difference between $F_m'$ and $F_s$; $\Delta F/F_m'$, effective quantum yield of PSII in light-acclimated state; FW, fresh weight; $PB_{tot}$, total phycobiliproteins (APC + PC + PE); PC, phycocyanin; PFD, photon flux density of actinic irradiance; PE, phycoerythrin

Seasonal development of macroalgae is generally triggered by light, temperature, and/or nutrient conditions (Chapman and Craigie 1977, Lüning 1980, Lüning and tom Dieck 1989). In Antarctic waters, sublittoral macroalgae are exposed to constant low temperatures (−1.8 to 2.0° C) and high nutrient concentrations (Clarke et al. 1988, Drew and Hastings 1992, Klöser et al. 1993). Their seasonal development depends mainly on variable light conditions and, especially, day length. Thus, assessment of the seasonal development of Antarctic species in long-term culture experiments is possible simply by simulating the seasonally fluctuating day lengths measured at the collecting site on King George Island, South Shetland Islands, Antarctica. Seasonal formation of gametes/spores and the induction of seasonal growth were monitored more closely than is possible in the field. The results of these studies complement the available fragmentary field observations and indicate that the phenology of Antarctic macroalgae can be controlled in the laboratory (Wiencke 1996). In such studies it has been demonstrated that growth of Antarctic macroalgae follows two different strategies to cope with the strong seasonality of the light regime. The sublittoral group and endemics such as *Palmaria decipiens* begin to grow and reproduce under short-day conditions in late winter/spring. The second group, mainly eulittoral and Antarctic cold temperate species, start growth later in favorable light conditions in spring and summer (Wiencke 1990a,b, Gómez et al. 1995, Gómez and Wiencke 1997, Weykam et al. 1997). The annual growth and reproduction of the first group, termed "season anticipators," appears to be controlled by photoperiodism and by circannual rhythms,

[1]Received 13 April 2001. Accepted 1 July 2002.

[2]Author for correspondence and present address: Monash University, School of Biological Sciences, P.O. Box 18, Victoria 3800, Australia; email: ulrike.lueder@monash.sci.edu.au

[3]Present address: Novozymes A/S, Research and Development, Detergent Applications, Krogshjvej 36, DK-2880 Bagsvaerd, Denmark.

triggered or synchronized by day length (Kain 1989, Wiencke 1996). The second group, the "season responders," react to changing environmental conditions, showing an opportunistic life strategy. In addition to seasonal growth, seasonal photosynthetic performance of some Antarctic species has also been studied in long-term culture studies (Weykam and Wiencke 1996, Gómez and Wiencke 1997, Lüder et al. 2001a).

Antarctic sublittoral algae are further effected by sea ice covering during winter months. Especially if the ice is covered with snow, dim light or complete darkness prevails in the sublittoral zone for up to 10 months (Zielinski 1990, Miller and Pearse 1991, Drew and Hastings 1992, Klöser et al. 1993). After the breakup of sea ice in Antarctic spring, the water is very clear, the sunlight penetrates deeply into the water column, and day length increases rapidly. In summer, the water becomes turbid as a result of plankton blooms and glacial melt water (Klöser et al. 1993). How do algae acclimate to this long period of darkness and to the sudden increase in irradiance in spring? Weykam et al. (1997) showed that winter sea ice cover could be simulated by interrupting the modulation of Antarctic day lengths with a period of 6 months of darkness, which would occur during the winter months (April–October).

We investigated the ecophysiology and life strategy of *P. decipiens* as one of the most common Antarctic season anticipators in more detail. *Palmaria decipiens* is endemic in the upper sublittoral zone of the Antarctic (Lamb and Zimmermann 1977, Ricker 1987). It is pseudo-perennial and develops new blades during late winter/early spring (Wiencke 1990b). Growth and photosynthesis are maximal in spring (October/November) (Wiencke 1990b, Weykam and Wiencke 1996, Lüder et al. 2001a). The light requirements for growth and photosynthesis of this species are very low (Wiencke 1990b, Wiencke et al. 1993, Weykam et al. 1996). Even in darkness, *P. decipiens* started to develop new blades in early August, supporting the theory of circannual rhythms for growth triggered or synchronized by day length (Weykam et al. 1997). Growth rates were low or even negative during darkness but were maximal in spring after re-illumination. Photosynthetic performance, measured as oxygen production, was reduced dramatically during darkness but also recovered in spring to maximal values. The long period of darkness was survived probably by using floridean starch accumulated in the previous summer.

Here our aim was to mimic exposure to darkness, simulating the influence of sea ice cover in winter. We investigated the acclimation processes of the photosynthetic apparatus to seasonally changing day lengths and darkness. Photosynthetic performance, measured by *in vivo* chl *a* fluorescence, and pigment content, in particular the phycobiliproteins, were determined monthly. In *P. decipiens* the phycobiliproteins, phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), are organized in so-called hemiellipsiodal phycobilisomes, the supramolecular structures of pigment–protein complexes that function in red algae and cyanobacteria as light-harvesting antennae (Lüder et al. 2001b). Changes in the phycobiliprotein ratios give further indications about changes in phycobilisome number and structure. In addition, a time course of acclimation to re-illumination in October was performed. The results give new insights into the life strategy of this alga.

## MATERIALS AND METHODS

*Algal material and culture conditions.* Tetraspores of *P. decipiens* (Reinsch) Ricker were collected from King George Island (South Shetland Islands, Antarctica, 62°12′S, 58°58′W) and developing male gametophytes were cultivated in the laboratory under seasonally fluctuating day lengths (mimicking field conditions) at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany (Wiencke 1988, 1990a). For 2 years several bunches of young male gametophytes were grown in 24, 3-L glass beakers under seasonally fluctuating day lengths under a constant irradiance of 25 μmol photons·m$^{-2}$·s$^{-1}$ using daylight fluorescent tubes (L58/W19, Osram, Munich, Germany) at a constant temperature of 0 ± 1° C in Provasoli enriched North Sea water (34 psu, pH 8, Provasoli 1968) aerated with pressurized air. To ensure sufficient nutrient supply, the culture medium was changed every second week.

During the first year, a biomass of about 1200 g fresh weight (FW) of *P. decipiens* was accumulated and acclimated to seasonal changing light conditions. At the beginning of the second year, in Antarctic March (14.3 h light per day), the 13-month experiment was begun and individual bunches were harvested from different beakers at monthly intervals for chl fluorescence, pigment, and dry weight (DW) measurements. From 2 April to 1 October the algae were exposed to darkness to simulate winter sea ice cover. Afterward, they were re-illuminated according to the day length that the species would experience in the field (2 October: 13 h light per day). Acclimation to light in October was investigated in more detail, and subsamples were taken after 1, 3, 5, 7, 10, 14, 17, 21, 24, and 28 days of re-illumination of dark-incubated plants.

*Chl fluorescence measurements. In vivo* chl *a* fluorescence was measured with a pulse-amplitude modulated fluorometer (PAM 2000, Walz, Effeltrich, Germany) connected to a personal computer after Schreiber et al. (1986, 1994).

The optimal quantum yield of PSII was determined as the ratio of variable to maximal fluorescence ($F_v/F_m$) of temporally dark-incubated algae according to Hanelt (1998) and Bischof et al. (1999). Thallus branches were fixed with a net close to the fiberoptic probe of the fluorometer and inserted into a custom-made metal cuvette filled with seawater at 0° C. First, a 5-s far red pulse, which selectively excites PSI (≈30 μmol photons·m$^{-2}$·s$^{-1}$, 735 nm) was applied to oxidize the electron transport chain, and then the algae were kept for a further 5 min in the cuvette in darkness. Subsequently, a pulsed dim red light (≈0.3 μmol photons·m$^{-2}$·s$^{-1}$, 650 nm) was applied to measure the minimal fluorescence ($F_o$; all reaction centers of PSII are oxidized or "open"), followed by a short pulse of saturating white light (0.6 s, ≈8000 μmol photons·m$^{-2}$·s$^{-1}$) to measure the maximal fluorescence ($F_m$; all reaction centers of PSII are reduced or "closed") of this temporarily dark-incubated sample. The variable fluorescence ($F_v$) was determined with the following equation: $F_v = F_m - F_o$ .Thus, the optimal quantum yield is given by $F_v/F_m = (F_m - F_o)/F_m$ .

Subsequently, a light response curve of fluorescence was recorded with increasing actinic red light irradiances of 3.5 to 350 μmol photons·m$^{-2}$·s$^{-1}$ (650 nm) and used to calculate the maximal electron transport rate ($ETR_{max}$). First, at each irradiance the effective quantum yield of PSII ($\Delta F/F_m'$) was determined as follows. The algae were temporarily irradiated with the first actinic red light irradiance (650 nm, 3.5 μmol photons·m$^{-2}$·s$^{-1}$), and after stabilization of the fluorescence level the steady-state fluorescence ($F_s$) was measured and a pulse of saturating white light applied to measure the maximal fluorescence of the light-incubated algae ($F_m'$), followed by 5 s of darkness to measure the

minimal fluorescence of the light-incubated algae ($F_o'$). Every 30 s, the actinic irradiance was further increased, and the respective $F_v$, $F_m'$, and $F_o'$ were determined. For each irradiance, the effective quantum yield of PSII ($\Delta F/F_m'$) was calculated using the following equation: $\Delta F/F_m' = (F_m' - F_s)/F_m'$ (Genty et al. 1989).

Second, for each irradiance the relative electron transport rate (ETR) was calculated by multiplying the effective quantum yield with the respective photon flux density of actinic irradiance (PFD): $ETR = \Delta F/F_m' \cdot PFD$ (Schreiber et al. 1994). Then, ETR was plotted against PFD (light response curve of ETR). The $ETR_{max}$ was calculated from the saturated part of the resulting curve (plateau) by linear regression analysis. PFD on front of the fiberoptics was measured using a quantimeter (Li-185B with a cosine-corrected irradiance quantum sensor Li 190 SA, Li-Cor, Lincoln, NE, USA).

All measurements were conducted in seawater at 0° C using 8–10 different individual bunches of *P. decipiens* from different beakers at the beginning of the light period. In macroalgae, the results of fluorescence measurements are consistent with those of oxygen measurements as shown earlier (Hanelt 1992, Hanelt et al. 1992, 1994). Thus, $ETR_{max}$ can be used as an indicator for maximal photosynthetic performance.

*Pigment determinations.* Phycobiliprotein contents were determined after Rosenberg (1981) in six different individual bunches of *P. decipiens* from different beakers. The algal material was ground in liquid nitrogen and extracted in 0.1 M potassium phosphate buffer, pH 6.8. Freezing and thawing several times optimized the extraction process. The resulting extract was gently centrifuged (800*g*, 4° C, 5 min), and the absorbance of the supernatant was determined photometrically at 565, 615, and 650 nm (UV-2101PC, Shimadzu, Kyoto, Japan). The phycobiliprotein concentrations were calculated using the following equations:

$$PE\ (\mu g \cdot mL^{-1}) = 123.5 \times OD_{565} - 73.5 - OD_{615} \times OD_{650}$$

$$PC\ (\mu g \cdot mL^{-1}) = 163.2 \times OD_{615} - 117.1 \times OD_{650}$$

$$APC\ (\mu g \cdot mL^{-1}) = 165.6 \times OD_{650} - 16.4 \times OD_{615}$$

Chl *a* was extracted in *N,N*-dimethylformamide using a modified method of Inskeep and Bloom (1985). Six to eight different individuals from different beakers were kept in 5 mL *N,N*-dimethylformamide at 4° C in the dark for 4 days. After gentle centrifugation (800*g*, 4° C, 5 min), the absorbance of the supernatant was determined photometrically at 664.5 nm (UV-2101PC, Shimadzu). The chl *a* concentration was calculated using the following equation: chl *a* ($mg \cdot L^{-1}$) = $12.70 \times OD_{664.5}$.

*FW and DW determinations.* For the determination of FW, 10 different individual bunches of *P. decipiens* were carefully blotted with paper tissue and weighed. After drying at 45° C for 24 h in an oven, they were weighed again. The DW was calculated as the percent ratio of DW to FW: DW:FW (%) = DW (g) × 100/FW (g).

*Regression analysis.* Regression analyses (linear, logarithmic, exponential) were performed to describe the relationship between pigment content and photosynthetic performance. The best fitting regression model was determined by comparing the coefficients of determination ($r^2$).

## RESULTS

*Palmaria decipiens* was exposed for 6 months to complete darkness to simulate Antarctic winter conditions (Fig. 1A). Values of $ETR_{max}$ were unaffected during the first 2 months of darkness but began to decrease in the third month of darkness. After 6 months of darkness, $ETR_{max}$ reached values close to zero (Fig. 1B). Values of $F_v/F_m$ decreased slightly (0.46–0.47) during the first 2 months of darkness and then drastically in the third month of darkness; by October $F_v/F_m$ was only 0.1 (Fig. 1B). In November, only 1 month after re-exposure to light, $ETR_{max}$ and $F_v/F_m$ were fully recovered. $F_v/F_m$ reached 0.59, and $ETR_{max}$ increased to the highest values obtained in this experiment. In the following summer months, $ETR_{max}$ decreased again, whereas $F_v/F_m$ continued to increase until February.

The pigments showed an interesting variation during darkness (Fig. 1C). After 1 month of darkness, the concentrations of chl *a* and to a lesser extent of PE were reduced, whereas PC increased slightly. However, during the following 2 months of darkness, an increase in chl *a* (from 77 to 120 $\mu g \cdot g^{-1}$ FW), PC (from 48 to 68 $\mu g \cdot g^{-1}$ FW), PE (from 183 to 250 $\mu g \cdot g^{-1}$ FW), and APC (from 27 to 30 $\mu g \cdot g^{-1}$ FW) was observed. In the fourth month of darkness all phycobiliproteins began to decrease drastically, whereas chl *a* began to decrease 1 month later. After 6 months of darkness the thalli appeared light green to white in correspondence with the lowest phycobiliprotein and very low chl *a* concentrations observed (10 μg APC, 9 μg PC, 50 μg PE, and 82 μg chl $a \cdot g^{-1}$ FW). After re-exposure to light, all pigments rapidly increased and the concentrations of phycobiliproteins were maximal in December (119 μg APC, 215 μg PC, and 1544 μg $PE \cdot g^{-1}$ FW); the concentration of chl *a* was also very high (183 $\mu g \cdot g^{-1}$ FW). The phycobiliproteins began decreasing in January, whereas chl *a* continued to increase.

The seasonal changes in the ratios of total phycobiliproteins ($PB_{tot}$) to chl *a* ($PB_{tot}$:chl *a*) are very similar to the changes in APC:chl *a* (Fig. 1D). In the first month of darkness $PB_{tot}$:chl *a* and APC:chl *a* increased slightly and began to fall from May onward until October. After re-exposure to light, both parameters increased and were maximal in December. The ratios of phycobiliproteins are shown in Figure 1E. During the months of darkness PE:APC and PC:APC ratios changed in the same way but opposite to that of PE:PC. During the first month of darkness PE:PC and PE:APC decreased, whereas PC:APC increased minimally. In the following month PC:APC and PE:APC increased. In June and July PE:PC was minimal, whereas PE:APC and PE:PC were maximal. In further darkness PE:APC and PC:APC both decreased, whereas PE:PC increased markedly. After re-exposure to light, PC:APC and PE:APC dramatically increased until January, whereas PE:PC began to decrease from December onward.

Seasonal changes in chl *a* and $PB_{tot}$ contents were plotted against changes in $F_v/F_m$ and $ETR_{max}$ (Fig. 2). Regression analysis was used to describe the relationship between pigment content and photosynthetic performance. The best fit between chl *a* content and $ETR_{max}$ and between chl *a* content and $F_v/F_m$ (when the data of the last 4 months in darkness were excluded) was a linear regression ($r^2 = 0.445$, Fig. 2A, and $r^2 = 0.768$, Fig. 2B, respectively). The relation between $PB_{tot}$ content and $F_v/F_m$ (Fig. 2D) could be

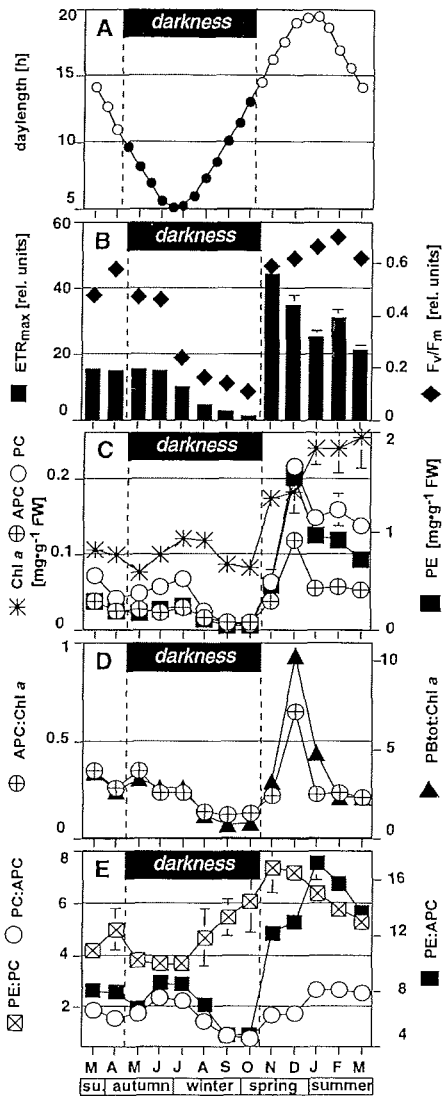


FIG. 1. Simulation of seasonal changes in (A) day length on King George Island, South Shetlands, Antarctica, including simulation of winter sea ice covering by exposure of *Palmaria decipiens* to darkness for 6 months (2 April to 1 October, marked by the "darkness" bar across the top of each panel). Seasonal changes in (B) $ETR_{max}$ and optimal quantum yield ($F_v/F_m$), (C) in pigment contents, (D) in ratios of $PB_{tot}$:chl *a* and APC:chl *a*, and (E) in ratios of PE:APC, PE:PC, and PC:APC. Means ± SD, $n$ = 8–10 (B); $n$ = 6–8 in (C) and (E); in those cases where no error bars are given, the SD is smaller than the symbol.

described with a logarithmic regression ($r^2$ = 0.771) and a linear regression ($r^2$ = 0.595) for the initial ascent of these curves. The relationship between $PB_{tot}$ content and $ETR_{max}$ could be described broadly with a logarithmic regression ($r^2$ = 0.917, Fig. 2C, inset), but we could also assume two linear regressions (Fig. 2C): one for the initial ascent of this curve ($r^2$ = 0.799) and one for the second part ($r^2$ = 0.922). Thus, during the entire year $F_v/F_m$ increased proportionally to the chl *a* content (when excluding the last 4 months of the dark period) and a positive correlation between chl *a* content and $F_v/F_m$ could be assumed. In contrast, $F_v/F_m$ increased initially proportionally to the $PB_{tot}$ content, but above a $PB_{tot}$ level of about 500 $\mu g \cdot g^{-1}$ FW a further increase led to only a small further rise in $F_v/F_m$. Thus, a positive correlation between $PB_{tot}$ content and $F_v/F_m$ could be assumed only under low $PB_{tot}$ levels (below about 500 $\mu g \cdot g^{-1}$ FW). Over the entire year, $ETR_{max}$ increased initially proportionally to $PB_{tot}$ content, but above a $PB_{tot}$ level of about 500 $\mu g \cdot g^{-1}$ FW a further increase led to a much lower rise in $ETR_{max}$, which could also be described as proportional. Thus, over the entire year $ETR_{max}$ increased in two steps proportional to the $PB_{tot}$ content and a positive correlation between $PB_{tot}$ content and $ETR_{max}$ could be assumed.

Seasonal changes in the percent ratio of DW:FW are shown in Figure 3. The DW:FW ratio was rather similar around 11.5% in May, June, September, and October during darkness but fell slightly to 10.6% in July followed by a marked peak of 13.4% in August. After re-exposure to light the DW:FW ratio increased to 16.6% in December, followed by a decrease until February/March to the value measured in the previous March.

The acclimation process of photosynthesis and pigments after re-exposure to light in October was investigated in more detail. On the first day of light, both $ETR_{max}$ and $F_v/F_m$ showed elevated values (Fig. 4A), and after a lag-phase of 5 days both parameters increased simultaneously. After 7 days of re-illumination, $ETR_{max}$ reached the value of the preceding April, before darkness (Fig. 1B). $F_v/F_m$ was fully recovered with a value of 0.59 24 days after re-illumination. The changes in pigment contents are shown in Figure 4B. On the first day of re-illumination, chl *a* increased above the value of the preceding April, before darkness began. All three phycobiliproteins began to increase slightly after 7 days of light, exceeded the values measured before darkness by day 15 or 16 of light, and showed a clear rise from day 17 onward.

Changes during the re-exposure to light in October in chl *a* and $PB_{tot}$ contents were plotted against changes in $F_v/F_m$ and $ETR_{max}$ (Fig. 5). Regression analyses were

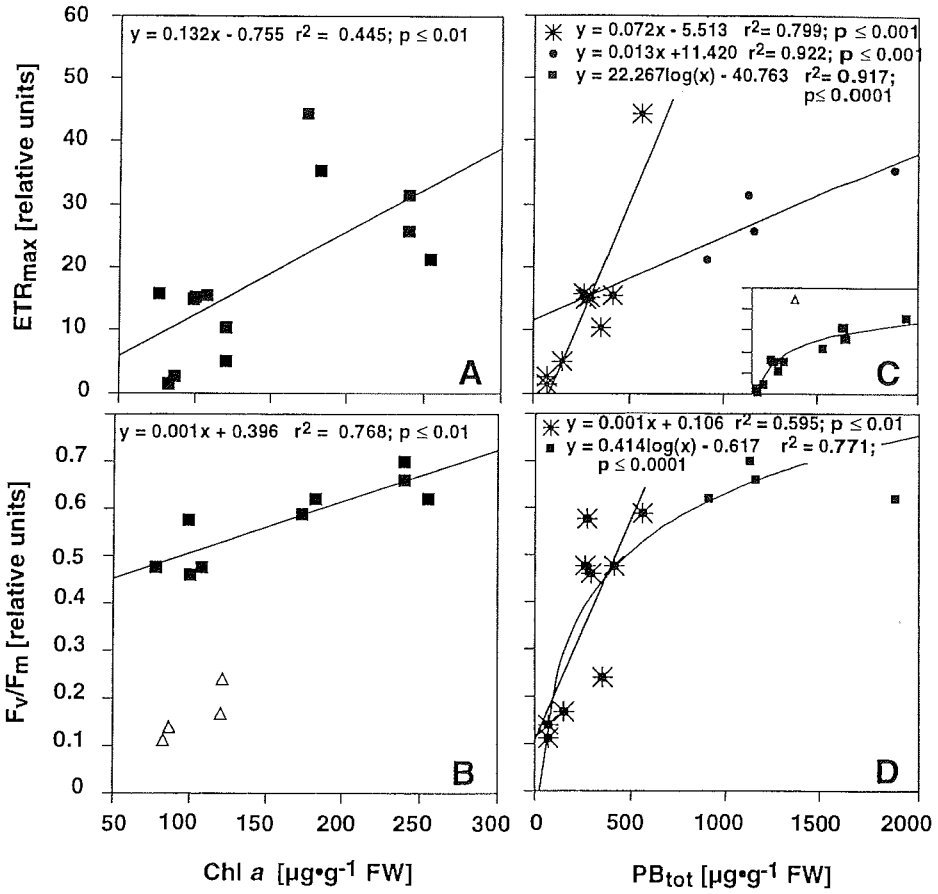


FIG. 2. Regressions between pigment contents and photosynthetic performance during a simulated Antarctic year including darkness. Triangle symbols in B were not used for the regression analysis; they correspond to data collected during the last 4 months of the dark period. The coefficients of determination ($r^2$) and probability ($p$) are given.

used to describe the relationship between pigment content and photosynthetic performance. Between chl *a* content and $ETR_{max}$ and between chl *a* content and $F_v/F_m$, the best fit was a linear regression ($r^2$ = 0.683, Fig. 5A, and $r^2$ = 0.774, Fig. 5B, respectively). The relationship between $PB_{tot}$ content and $ETR_{max}$ and between $PB_{tot}$ content and $F_v/F_m$ could be described with a logarithmic regression ($r^2$ = 0.937 and $r^2$ = 0.937, respectively); for the initial ascent of these curves we found a linear regression ($r^2$ = 0.952, Fig. 5C, and $r^2$ = 0.903, Fig. 5D, respectively). Thus, $F_v/F_m$ and $ETR_{max}$ increased proportionally to the chl *a* content during re-illumination in October. In contrast, $F_v/F_m$ and $ETR_{max}$ increased initially proportionally to the $PB_{tot}$ content, but above a $PB_{tot}$ level of about 250 µg·g$^{-1}$ FW a further increase led to no further rise in $ETR_{max}$ and $F_v/F_m$. Thus, a positive correlation between $PB_{tot}$ content and $F_v/F_m$ and between $PB_{tot}$ content and $ETR_{max}$ could be assumed only under $PB_{tot}$ level below 250 µg·g$^{-1}$ FW.

## DISCUSSION

*Acclimation to darkness.* The maximal photosynthetic performance of *P. decipiens* remained unchanged during the first 2 months of darkness, and even $F_v/F_m$ was

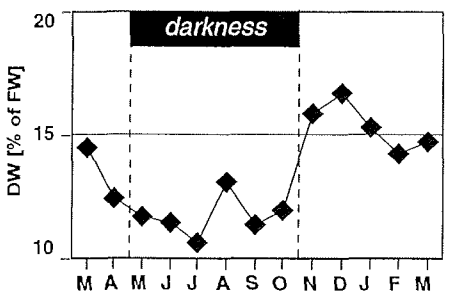


FIG. 3. Seasonal changes in the percent ratio of dry weight to fresh weight (DW:FW). Means ± SD, $n = 10$; in those cases where no error bars are given, the SD is smaller than the symbol.

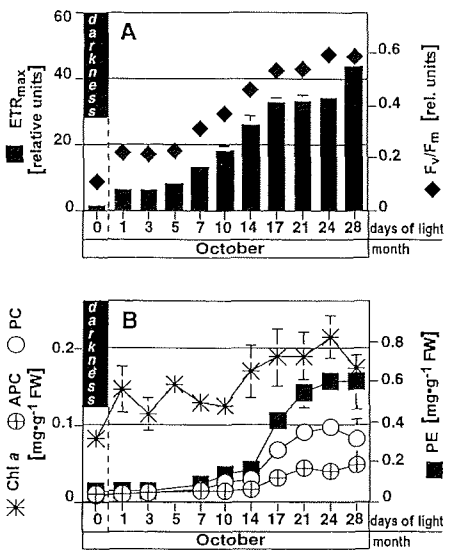


FIG. 4. Re-illumination of dark-incubated *Palmaria decipiens* in October. Acclimation of (A) $ETR_{max}$ and optimal quantum yield ($F_v/F_m$) and of (B) chl *a* and phycobiliprotein contents. The "darkness" bar at the left of each panel marks the last value measured of dark-incubated algae. Means ± SD, $n = 8$–10 (A), $n = 6$ (B); in those cases where no error bars are given, the SD is smaller than the symbol.

reduced only slightly. The clear reduction of both parameters after the third month of darkness may be interpreted as the start of the degradation of the photosynthetic apparatus. The phycobiliprotein levels started to decrease after 4 months and the chl *a* levels after 5 months of darkness. In contrast, Weykam et al. (1997) observed a gradual decrease of photosynthetic oxygen production during darkness in a similar long-term culture study with *P. decipiens* and found no major changes in chl *a* (per FW); phycobiliproteins were not measured. One reason for differences in comparison with the present study may be the difference in age of the algae. In Weykam et al.'s study, all measurements were performed on only one gametophyte, which was several years old, whereas in this study many 2-year-old individuals were used. Nevertheless, both studies showed that after 6 months of darkness *P. decipiens* lost its ability to photosynthesize.

The present study shows that the PE and PC content increased continuously during the first 3 months of darkness. In contrast, chl *a* showed an initial reduction followed by an increase. A rise in pigments during the first period of darkness has also been observed in other macroalgae (Sheath et al. 1977, Bird et al. 1982) and in microalgae (Peters 1996, Peters and Thomas 1996). Sheath et al. (1977) further demonstrated that PE and chl *a* can be synthesized in darkness at rates comparable with those in the light. Increases in pigment concentrations during the first period of darkness seem to be comparable with photoacclimation processes to low light, but a prolongation over 3 months of darkness is unusual and may be a feature of the life strategy of *P. decipiens*. In a parallel year-round study without exposure to darkness (Lüder et al. 2001a), the seasonal changes in phycobiliprotein and chl *a* content showed a continuous rise in mid-autumn and winter and were highest in spring; therefore, a photoperiodic control of pigment synthesis triggered by day length was discussed, as previously suggested for growth in *P. decipiens* (Wiencke 1990b, 1996, Weykam and Wiencke 1996). Thus, the observed rise in pigments during the first 3 months of darkness also suggests that the pigment synthesis follows a fixed seasonal pattern and supports the hypothesis of a photoperiodic control of pigment synthesis or even an underlying circannual rhythm.

The phycobiliprotein ratios may give further indication of changes in phycobilisome number and size/structure. In general, phycobilisome number and size (number of rods, rod length) can change according to acclimation processes in response to light intensity, light quality, or nutrient availability (Gantt 1990, Grossman et al. 1994, Talarico 1996). The decrease in PE:APC and PC:APC ratios suggests the loss of whole rods from the phycobilisomes during the last 3 months of the dark period. The increase of the PE:PC ratio during this period suggests an extension of the remaining rods, which is unexpected. Instead, we propose that the increased PE:PC ratio is due to an excess of free PE, detached from the periphery of the phycobilisomes that might appear in a free pool of PE to be subsequently degraded as previously discussed by Algarra and Rüdiger (1993). Even Wyman et al. (1985) found an excess of the PE:PC ratio in a marine PE-containing cyanobacterium and showed that most of the absorbed light energy was lost as PE autofluorescence. The accumulation of this energetically uncoupled PE

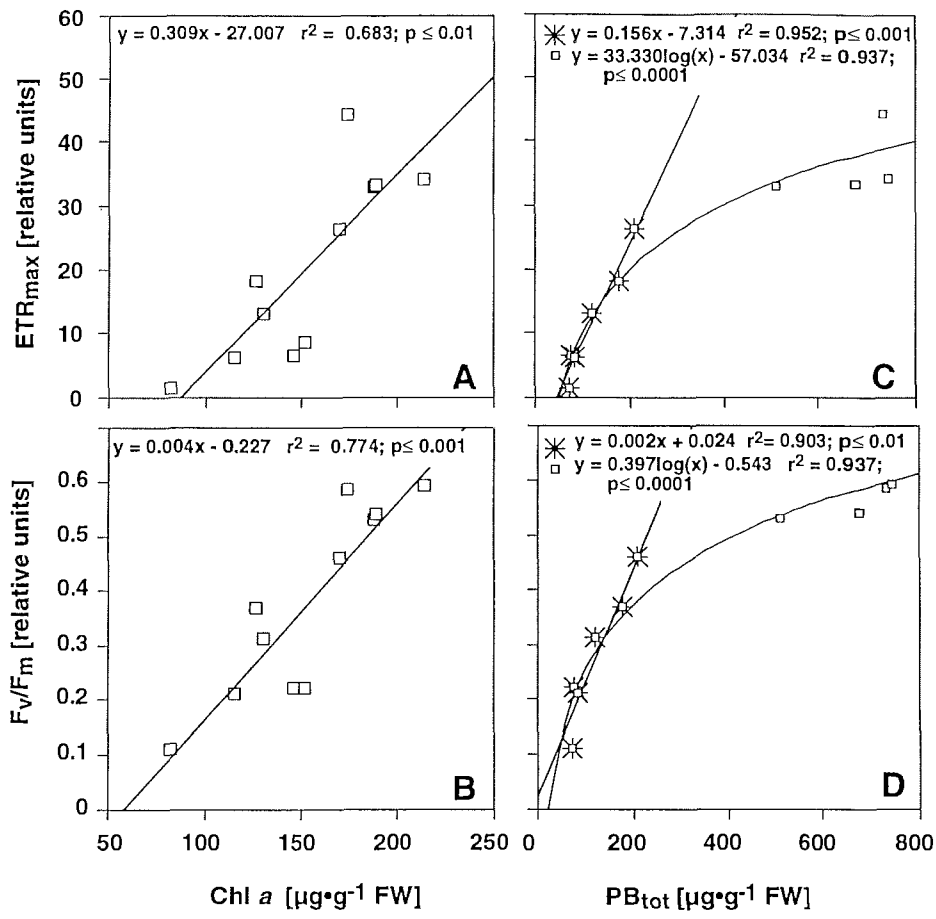


FIG. 5. Regressions between pigment contents and photosynthetic performance during re-illumination of dark-incubated *Palmaria decipiens* in October. The coefficients of determination ($r^2$) and probability ($p$) are given.

was suggested to function as nitrogen reserve or even as photoprotection again excess light. The observed decrease in APC content during the dark period of our study was most probably due to a degradation of whole phycobilisomes. The degradation of phycobilisomes was described as an ordered process, catalyzed by a protease, whereby the remaining "small" phycobilisomes still function in harvesting light energy (Grossman et al. 1994).

The reduction of chl *a* levels during the last 2 months of darkness might point to a degradation of PSII and PSI reaction centers. Moreover, PSI possesses a small antenna, mainly consisting of chl *a*, which may also decrease in size and/or number. Nevertheless, the remaining chl *a* content was still considerably high when compared with the chl *a* content measured before the period of darkness. So we assume that the extremely low $F_v/F_m$ value of 0.1 indicates a damage of the reaction center of PSII. The biodegradation and *de novo* synthesis of D1 protein (the half-time of D1 protein turnover can be as short as 30 min; Aro et al. 1993) is probably disturbed, perhaps through a lack of amino acids. Moreover, the synthesis of D1 protein

itself is light dependent, and it is well known that only limited repair of photodamaged PSII complexes occurs in darkness (Melis 1991, Aro et al. 1993).

*Palmaria decipiens* developed new blades in June during darkness, corresponding to the start of the seasonal growth period (Wiencke 1990b). Weykam et al. (1997) observed new blade formation in *P. decipiens* a little later in early August. The energy required to survive darkness and even to accumulate pigments and to start blade formation was obtained from stored floridean starch, accumulated during the previous summer (Weykam et al. 1997). One reason for the large decrease in phycobiliproteins from August onward may be that floridean starch had become a limiting factor and that *P. decipiens* requires the remaining energy to support growth at the expense of photosynthesis. This is supported by a significant decrease in floridean starch content observed in August by Weykam et al. (1997). Furthermore, the phycobiliproteins themselves may be used as a nitrogen reserve to ensure growth (Bird et al. 1982, Wyman et al. 1985, Grossman et al. 1994). Another alternative energy source may be the degradation of thylakoids, as observed during dark incubation by Sheath et al. (1977) and Lüning and Schmitz (1988). But the chl *a* level in *P. decipiens* was still considerably high at the end of the dark period, which would exclude this option.

Nevertheless, the percent ratio of DW:FW was rather similar during darkness, although a slight fall of about 1% in July followed by a marked increase of about 2.5% in August was observed. Weykam et al. (1997) measured rather constant DW:FW ratios during darkness in a similar study with *P. decipiens*, but the data also showed a rise of about 1.7% in August. Thus, this August peak is reproducible. The simplest explanation would be a decrease in water content. This would also agree with the negative growth rate (determined as daily increase in FW) measured in August in Weykam et al.'s study.

July/August seems to be the time when physiology changes strongly during darkness. New blades are formed, the content of floridean starch and of phycobiliproteins drops, and the DW:FW ratio increases, but it is a period of increasing day length. Thus, these physiological changes might be related to the annual growth pattern under photoperiodic control or may even have an underlying circannual rhythm, possibly triggered by day length (Wiencke 1996). A short-day response, such as observed in many other red and brown macroalgae (Kain and Norton 1990, Lüning 1990), would also be possible.

*Acclimation to re-illumination.* The more detailed study of re-exposure to light showed that the chl *a* accumulation (= synthesis?) begins in 24 h or less after re-illumination, whereas the phycobiliprotein accumulation (= synthesis?) began very slowly with a delay time of about 7 days. Thus, the initial increase of $F_v/F_m$ and $ETR_{max}$ 24 h after re-illumination can be due only to the chl *a* increase. A similar rapid chl *a* synthesis was observed in *Delesseria sanguinea* (Ceramiales, Rhodophyta) exposed to darkness for 9 months (Lüning and Schmitz 1988). Even here the phycobiliprotein content remained unchanged during the first week of re-illumination, but in contrast photosynthetic oxygen production was first detected after 3 days of re-illumination. A similar rapid recovery of photosynthetic performance was found in dark-incubated (3 weeks) *Porphyra leucosticta* (Bangiales, Rhodophyta) after 1 day of re-illumination (Sheath et al. 1977).

The further increase of $ETR_{max}$ to its initial value (before darkness begun) at day 7 of re-illumination may point to the slight increase of phycobiliprotein contents. Nevertheless, after 17 days of re-illumination a marked increase of phycobiliproteins occurred and $ETR_{max}$ reached a value equivalent to the maximum found in a parallel simulation study without a 6-month dark period (Lüder et al. 2001a).

A strong relationship between pigment content and photosynthetic performance was evident with a positive correlation between chl *a* content and $ETR_{max}$ and between chl *a* content and $F_v/F_m$ during re-illumination of *P. decipiens*. After the onset of photosynthesis on day 3 of re-illumination, Lüning and Schmitz (1988) also observed a parallel increase of chl *a* content and photosynthetic oxygen production during the first week of re-illumination of *D. sanguinea* (Ceramiales, Rhodophyta).

In contrast, the relationship between $PB_{tot}$ content and $ETR_{max}$ and between $PB_{tot}$ and $F_v/F_m$ during re-illumination of *P. decipiens* followed a logarithmic form. Further, a positive correlation between $PB_{tot}$ content and $ETR_{max}$ and between $PB_{tot}$ content and $F_v/F_m$ could be described only at low $PB_{tot}$ contents (below about 250 $\mu g \cdot g^{-1}$ FW). This suggests that above a certain level of phycobiliproteins, a further increase leads to no further rise in $ETR_{max}$ and $F_v/F_m$. For $F_v/F_m$ this is logical as long as the value cannot increase above 0.6–0.7 in red algae (Büchel and Wilhelm 1993). Talarico (1996) also observed in *Audouinella saviana* (Nemaliales, Rhodophyta) that maximum pigment content does not always necessarily mean maximum photosynthethic activity and concluded that not all available PE functions to optimize photosynthesis. This would occur only in the presence of well-assembled phycobilisomes, and phycobiliproteins are not always organized in well-assembled phycobilisomes (Lüning and Schmitz 1988, Foltran et al. 1996, Talarico 1996). During an annual cycle in *Halopithys incurvus* (Ceramiales, Rhodophyta) well-assembled phycobilisomes were found at low phycobiliprotein levels, whereas small and incompletely assembled phycobilisomes occurred at high levels (Talarico and Kosovel 1983). Furthermore, an accumulation of PE in excess of its requirement for this phycobiliprotein as a light-harvesting pigment was demonstrated in *Porphyridium* sp. (Porphyridiales, Rhodophyta; Sivan and Arad 1993) and in *Gracilaria tikvahiae* (Gracilariales, Rhodophyta; Ramus and van der Meer 1983). There, the PE-deficient mutants achieved similar or even higher photosynthetic rates than their wild types. A similar situation was seen

in a PE-containing marine cyanobacterium in nitrogen-sufficient cultures when compared with nitrogen-limited cultures (Wyman et al. 1985). A function of the excess PE as a nitrogen reserve, or even as photoprotection, was suggested by Wyman et al. (1985). Foltran et al. (1996) discussed a function as storage material readily available for phycobilisome formation when adaptive response to a reduced light intensity is required. We also believe that additional PE in *P. decipiens* might be stored as a nitrogen reserve and could also be used in winter to survive darkness when stored floridean starch becomes limited. A storage role for phycobiliproteins would also explain why a further increase of phycobiliproteins was seen in December, while $ETR_{max}$ started to decrease. Furthermore, the concentration of PE was twice as high at this time than the maximal value measured in a parallel simulated study without darkness (Lüder et al. 2001a), whereas the concentrations of APC and PC were only slightly higher. Even the regression analysis over the entire year showed that $ETR_{max}$ increased in two steps proportional to the $PB_{tot}$ content, whereby at higher $PB_{tot}$ contents the positive effect of $ETR_{max}$ is much lower than at lower $PB_{tot}$ contents.

A decrease of $ETR_{max}$ and of the phycobiliprotein contents during the following summer was also observed in *P. decipiens* in a parallel simulation study without a 6-month dark period (Lüder et al. 2001a) and suggests a degradation of the photosynthetic apparatus. A photoinhibitory response of *P. decipiens* to strong light was previously shown by Hanelt et al. (1994). Because *P. decipiens* is adapted to very low light (Wiencke 1990b, Wiencke et al. 1993, Weykam et al. 1996), a degradation of light-harvesting antennae might offer protection from photodamage caused by prolonged day length. A decrease of phycobiliproteins during summer was also found in *Gracilaria verrucosa* (Kosovel and Talarico 1979). Furthermore, a decrease in phycobiliproteins seems to be part of a normal photoacclimation response to high light (Levy and Gantt 1988, Cunningham et al. 1989, Sagert and Schubert 2000, Talarico and Maranzana 2000).

We conclude that *P. decipiens* is very well adapted to a prolonged dark period and to the short period of favorable light conditions experienced in the field. After 6 months of darkness *P. decipiens* loses its ability to photosynthesize with the degradation of its light-harvesting antennae, the phycobilisomes, and probably through degradation of the reaction centers of PSII. Nevertheless, within 24 h after re-illumination, *P. decipiens* starts to accumulate chl *a* and to photosynthesize, indicating rapid repair mechanisms. The phycobiliprotein accumulation (which may be equal to synthesis) starts with a time lag of about 7 days. The recovery of the photosynthetic performance is accelerated by the accumulation of pigments and buildup of new light-harvesting antennae expressed in the correlation found between pigment contents and $F_v/F_m$ and $ETR_{max}$ during the re-illumination. Likewise, after 7 days of re-illumination *P. decipiens* reaches $ETR_{max}$ values comparable with the values before darkness and maximal values after 30 days of re-illumination in November. This maximum correlates well with very clear water when sunlight penetrates deep into the water column in the field, after breakup of sea ice and before the water becomes turbid due to plankton blooms and glacial melt water. In summer, *P. decipiens* reduces its photosynthetic performance and pigment contents, probably to avoid photodamage caused by excess light energy. These results confirm that *P. decipiens* has a life strategy as a season anticipator.

Supported by grants from the Deutsche Forschungsgemeinschaft. We gratefully thank C. Langreder for excellent technical support. We acknowledge Richard Crawford for improvements to the English of this manuscript.

Algarra, P. & Rüdiger, W. 1993. Acclimation processes in the light harvesting complex of the red alga *Porphyridium purpureum* (Bory) Drew et Ross, according to irradiance and nutrient availability. *Plant Cell Environ.* 16:149–59.

Aro, E. M., Virgin, I. & Andersson, B. 1993. Photoinhibition of photosystem II. Inactivation, protein damage and turnover. *Biochem. Biophys. Acta* 1143:113–34.

Bird, K. T., Habig, C. & DeBusk, T. 1982. Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). *J. Phycol.* 18:344–48.

Bischof, K., Hanelt, D. & Wiencke, C. 1999. Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. *Plant Biol.* 1:435–44.

Büchel, C. & Wilhelm, C. 1993. In vivo analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. *Photochem. Photobiol.* 58:137–48.

Chapman, A. R. O. & Craigie, J. S. 1977. Seasonal growth in *Laminaria longicruris*: relation with dissolved inorganic nutrients and internal reserves of nitrogen. *Mar. Biol.* 40:197–205.

Cunningham, F. X., Jr., Dennenberg, R. J., Mustardy, L., Jutsinic, P. A. & Gantt, E. 1989. Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga *Porphyridium cruentum* as a function of growth irradiance. *Plant Physiol.* 91:1179–87.

Clarke, A., Holmes, L. J. & White, M. G. 1988. The annual cycle of temperature, chlorophyll and major nutrients at Signy Island, South Orkney Island, 1969–82. *Br. Antarct. Surv. Bull.* 80:65–86.

Drew, E. A. & Hastings, R. M. 1992. A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. *Phycologia* 31:262–77.

Foltran, A., Maranzana, G., Rascio, N., Scarabel, L., Talarico, L. & Andreoli, C. 1996. *Iridaea cordata* from Antarctica: an ultrastructural, cytochemical and pigment study. *Bot. Mar.* 39:533–41.

Gantt, E. 1990. Pigmentation and photoacclimation. *In* Cole, K. M. & Sheath, R. G. [Eds.] *Biology of the Red Algae.* Cambridge University Press, Cambridge, pp. 203–19.

Genty, B., Briantais, J. M. & Baker, N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochim. Biophys. Acta* 990:87–92.

Cómez, I. & Wiencke, C. 1997. Seasonal growth and photosynthetic performance of the Antarctic macroalga *Desmarestia menziesii* (Phaeophyceae) cultivated under fluctuating Antarctic daylengths. *Bot. Acta* 110:25–31.

Gómez, I., Wiencke, C. & Weykam, G. 1995. Seasonal photosynthetic characteristics of *Ascoseira mirabilis* (Ascoseirales, Phaeophyceae) from King George Island, Antarctica. *Mar. Biol.* 123:167–72.

Grossman, A. R., Schaefer, M. R., Chiang, G. G. & Collier, J. L. 1994. The responses of cyanobacteria to environmental conditions: light and nutrients. *In* Bryant, D. A. [Ed.] *The Molecular Biology of Cyanobacteria.* Kluwer Academic Publishers, Dordrecht, pp. 641–75.

Hanelt, D. 1992. Photoinhibition of photosynthesis in marine macrophytes of the South Chinese Sea. *Mar. Ecol. Prog. Ser.* 82:199–206.

Hanelt, D. 1998. Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. *Mar. Biol.* 131:361–9.

Hanelt, D., Jaramillo, J., Nultsch, W., Senger, S. & Westermeier, R. 1994. Photoinhibition as a regulative mechanism of photosynthesis in marine algae of Antarctica. *Ser. Cient. Inst. Antart. Chil.* 44:67–77.

Hanelt, D., Huppertz, K. & Nultsch, W. 1992. Photoinhibition of photosynthesis and its recovery in red algae. *Bot. Acta* 105:278–84.

Inskeep, W. P. & Bloom, P. R. 1985. Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80% acetone. *Plant Physiol.* 77:483–5.

Kain, J. M. 1989. The seasons in the subtidal. *Br. Phycol. J.* 24:203–15.

Kain, J. M. & Norton, T. A. 1990. Marine ecology. *In* Cole, K. M. & Sheath, R. G. [Eds.] *Biology of the Red Algae.* Cambridge University Press, Cambridge, pp. 377–422.

Klöser, H., Ferreyra, G., Schloss, I., Mercuri, G., Laturnus, F. & Curtosi, A. 1993. Seasonal variation of algal growth conditions in sheltered Antarctic bays: the example of Potter Cove (King George Island, South Shetlands). *J. Mar. Syst.* 4:289–301.

Kosovel, V. & Talarico, L. 1979. Seasonal variations of photosynthetic pigments in *Gracilaria verrucosa* (Huds.) Papenfuss (Florideophyceae-Gigartinales). *Boll. Soc. Adr. Sci.* 63:5–15.

Lamb, I. M. & Zimmermann, M. H. 1977. Benthic marine algae of the Antarctic Peninsula. *Antarct. Res. Ser.* 23:129–229.

Levy, I. & Gantt, E. 1988. Light acclimation in *Porphyridium purpureum* (Rhodophyta): Growth, photosynthesis, and phycobilisomes. *J. Phycol.* 24:452–8.

Lüder, U. H., Knoetzel, J. & Wiencke, C. 2001a. Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. *Polar Biol.* 24:598–603.

Lüder, U. H., Knoetzel, J. & Wiencke, C. 2001b. Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae). *Physiol. Plant.* 112:572–81.

Lüning, K. 1980. Control of algal life-history by daylength and temperature. *In* Price, J. H., Irvine, D. E. G. & Farnham, W. F. [Eds.] *The Shore Environment.* Vol. 2. *Ecosystems.* Academic Press, New York, pp. 915–45.

Lüning, K. 1990. *Seaweeds. Their Environment, Biogeography and Ecophysiology.* Wiley & Sons, New York, 527 pp.

Lüning, K. & Schmitz, K. 1988. Dark growth of the red alga *Delesseria sanguinea* (Ceramiales): lack of chlorophyll, photosynthetic capability and phycobilisomes. *Phycologia* 27:72–7.

Lüning, K. & tom Dieck, I. 1989. Environmental triggers in algal seasonality. *Bot. Mar.* 32:389–97.

Melis, A. 1991. Dynamics of photosynthetic membrane composition and function. *Biochim. Biophys. Acta* 1058:87–106.

Miller, K. A. & Pearse, J. S. 1991. Ecological studies of seaweeds in McMurdo Sound, Antarctica. *Am. Zool.* 31:35–48.

Peters, E. 1996. Prolonged darkness and diatom mortality. II. Marine temperate species. *J. Exp. Mar. Biol. Ecol.* 207:43–58.

Peters, E. & Thomas, D. N. 1996. Prolonged darkness and diatom mortality. I. Marine Antarctic species. *J. Exp. Mar. Biol. Ecol.* 207:25–41.

Provasoli, L. 1968. Media and prospects for the cultivation of marine algae. In Watanabe, A. & Hattori, A. [Eds.] *Cultures and Collections of Algae.* Proc. U.S.–Japan Conf., Hakone, Sept. 1966, *Jpn. Soc. Plant Physiol.*, pp. 63–75.

Ramus, J. & van der Meer, J. P. 1983. A physiological test of the theory of complementary chromatic adaptation. I. Color mutants of a red seaweed. *J. Phycol.* 19:86–91.

Ricker, R. W. 1987. *Taxonomy and Biogeography of Macquarie Island Seaweeds.* British Museum (Natural History), London, 344 pp.

Rosenberg, G. 1981. *Ecological Growth Strategies in the Seaweeds* Gracillaria foliifera *(Rhodophyceae) and* Ulva *sp. (Chlorophyceae).* Ph.D. thesis, Yale University, New Haven, Connecticut, 156 pp.

Sagert, S. & Schubert, H. 2000. Acclimation of *Palmaria palmata* (Rhodophyta) to light intensity: comparison between artificial and natural light fields. *J. Phycol.* 36:1119–28.

Schreiber, U., Bilger, W. & Neubauer, C. 1994. Chlorophyll fluorescence as a noninstrusive indicator for rapid assessment of in vivo photosynthesis. *In* Schulze, E. D. & Caldwell, M. M. [Eds.] *Ecophysiology of Photosynthesis* (series: Ecological Studies, volume 100). Springer, Berlin, pp. 49–70.

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

Sheath, R. G., Hellebust, J. A. & Sawa, T. 1977. Changes in plastid structure, pigmentation and photosynthesis of the conchocelis stage of *Porphyra leucosticta* (Rhodophyta, Bangiophyceae) in response to low light and darkness. *Phycologia* 16:265–76.

Sivan, A. & Arad, S. 1993. Induction and characterization of pigment mutants in the red microalga *Porphyridium* sp. (Rhodophyceae). *Phycologia* 32:68–72.

Talarico, L. 1996. Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. *Sci. Mar.* 60:205–22.

Talarico, L. & Maranzana, G. 2000. Light and adaptive responses in red macroalgae: an overview. *J. Photochem. Photobiol. B Biol.* 56:1–11.

Talarico, L. & Kosovel, V. 1983. Ficobilisomi e variazioni delle ficobiliproteine in *Halopithys incurvus* (Huds.) Batt. (Rhodomelaceae-Rhodophyta) durante il ciclo annuale. *Boll. Soc. Adr. Sci.* 67:69–81.

Weykam, G., Gómez, I., Wiencke, C., Iken, K. & Klöser, H. 1996. Photosynthetic characteristics and C:N ratios of macroalgae from King George Island (Antarctica). *J. Exp. Mar. Biol. Ecol.* 204:1–22.

Weykam, G., Thomas, D. N. & Wiencke, C. 1997. Growth and photosynthesis of the Antarctic red algae *Palmaria decipiens* (Palmariales) and *Iridaea cordata* (Gigartinales) during and following extended periods of darkness. *Phycologia* 36:395–405.

Weykam, G. & Wiencke, C. 1996. Seasonal photosynthetic performance of the endemic Antarctic alga *Palmaria decipiens* (Reinsch) Ricker. *Polar Biol.* 16:357–61.

Wiencke, C. 1988. Notes on the development of some benthic marine macroalgae of King George Island, Antarctica. *Ser. Cient. Inst. Antart. Chil.* 37:23–47.

Wiencke, C. 1990a. Seasonality of brown macroalgae from Antarctica—a long-term culture study under fluctuating Antarctic daylengths. *Polar Biol.* 10:589–600.

Wiencke, C. 1990b. Seasonality of red and green macroalgae from Antarctica—a long-term culture study under fluctuating Antarctic daylengths. *Polar Biol.* 10:601–7.

Wiencke, C. 1996. Recent advances in the investigation of Antarctic macroalgae. *Polar Biol.* 16:231–40.

Wiencke, C., Rahmel, J., Karsten, U., Weykam, G. & Kirst, G. O. 1993. Photosynthesis of marine macroalgae from Antarctica: light and temperature requirements. *Bot. Acta* 106:78–87.

Wyman, M., Gregory, R. P. F. & Carr, N. G. 1985. Novel role for phycoerythrin in a marine cyanobacterium *Synechoccus* DC2. *Science* 230:818–20.

Zielinski, K. 1990. Bottom macroalgae of the Admiral Bay (King George Island, South Shetlands, Antarctica). *Pol. Polar Res.* 11:95–131.

## *Publication 5*

Lüder UH, Knoetzel J and Wiencke C:

**Dynamics of phycobilisome assembly during and after six months of darkness in *Palmaria decipiens* (Florideophyceae) - a study to simulate Antarctic winter sea ice cover.**

(in preparation to be submitted).

# Dynamics of phycobilisome assembly during and after six month of darkness in *Palmaria decipiens* - a study to simulate Antarctic sea-ice cover

Ulrike H. Lüder [1,2], Jürgen Knoetzel [2*], Christian Wiencke [1]

[1] Foundation Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, D-27570 Bremerhaven, Germany

[2] Institute of Cell Biology, Biochemistry and Biotechnology, University of Bremen, Postbox 33 04 40, D-28334 Bremen, Germany

[*] present address
Novozymes A/S, Research and Development, Detergent Applications, Krogshjvej 36, DK-2880 Bagsvaerd, Denmark

Running title: phycobilisome degradation

## ABSTRACT

The influence of seasonally fluctuating Antarctic daylengths, of prolonged darkness simulating winter sea-ice cover and of subsequent re-exposure to light on the assembly of phycobilisomes was studied in *Palmaria decipiens* (Palmariales, Florideophyceae) in a long-term culture experiment, mimicking the Antarctic year. Phycobilisomes are the main light-harvesting antennae in red algae, consisting of phycobiliproteins, which are connected by linker polypeptides. In monthly intervals, intact phycobilisomes were isolated on discontinuous sucrose gradients and changes in their phycobiliprotein contents and in their polypeptide composition, analysed by SDS-PAGE, were determined. Two phycobilisome forms, $PBS_{up}$ and $PBS_{low}$, were found more or less before and after the dark period. From the second months of darkness on, $PBS_{low}$ completely disappeared. After one month of darkness, $PBS_{low}$ showed still constant PE:APC, PE:PC and PC:APC ratios, indicating no alteration in the phycobilisome size. The amount of remaining $PBS_{up}$ started to decrease after four months of darkness. The phycobilisome size of $PBS_{up}$ seemed to remain unaltered over the first five months of darkness, since more or less constant ratios of PE:APC, PE:PC and PC:APC were found. In the last month of darkness a degradation of the remaining $PBS_{up}$ occurred by uncoupling of hole rods. The rod loss was induced by the loss of PC and its associated rod core linker $L_{RC}^{30.6PC}$ and rod linker $L_R^{38.9PC}$, furthermore the $\gamma^{40.3}$-subunit was reduced. The $\gamma^{33.5}$-subunits did not vary during darkness. The remaining $\gamma^{37.3}$-subunit became much more dominant in the last two months of darkness.

After one months of re-illumination, $PBS_{up}$ was fully recovered. Moreover the phycobilisome size increased strongly by coupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. $PBS_{low}$ appeared again and also increased in size by coupling of the $\gamma^{33.5}$-subunits and its associated PE hexameter, but the coupling of $L_{RC}^{30.6PC}$ and of $L_R^{38.9PC}$ and their associated PC trimers was not recovered before February. This suggested the present of rods mainly consistent of PE and the direct coupling of the $\gamma^{33.5}$-subunits to APC.

However, in the Antarctic summer months (January-March), the amount of the $\gamma^{33.5}$-subunit decreased, indicating a reduction in phycobilisome size by uncoupling of some PE, both in $PBS_{up}$ and $PBS_{low}$.

## ABBREVIATIONS

APC, allophycocyanin; $L_C$ , core linker; $L_{CM}$ , core membrane linker; $L_R$, rod linker; $L_{RC}$, rod-core linker; PBS, phycobilisome; $PBS_{up}$ and $PBS_{low}$, upper and lower banded PBS, isolated on discontinuous sucrose gradients in *P. decipiens*; PC, phycocyanin; PE, phycoerythrin.

## INTRODUCTION

Phycobilisomes are the main light-harvesting antennae in red algae. (reviewed in Gantt 1990, Mörschel 1991, Reuter and Müller 1993, Sidler 1994, Bald et al. 1996, MacColl 1998, van Thor et al. 1998). They are consist of phycobiliproteins, which are connected by linker polypeptides. Phycobiliproteins are universally composed of α- and β-polypeptide subunits in equimolar (1:1) stoichiometry with one or more chromophores covalently attached (Stadnichuk 1995). They are organised *in vivo* in $(\alpha\beta)_3$ trimers and in $(\alpha\beta)_6$ hexamers, which interact with specific linker polypeptides to form disc-shaped units. This units form the two main structural elements of phycobilisomes: the tricylindrically core in the centre and several peripheral cylindrical rods. In red algae, the common phycobiliproteins are phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC). PE is located at the periphery of the rods, PC at the inner part of the rods and APC in the core. Linker polypeptides are divided into four groups, according to their structural function in the phycobilisome (Glazer 1985, Reuter and Müller, 1993): the core linker ($L_C$) assembles the core structure; the core membrane linker ($L_{CM}$) attaches the phycobilisome to the thylakoid membrane and coordinates the assembly of APC trimers; the rod-core linker ($L_{RC}$) attaches the rods to the core; the rod linkers ($L_R$) are involved in the assembly of the peripheral rods. γ-subunits are coloured linkers and were originally found only in PE of red algae (Glazer and Hixon 1977). More recent studies have demonstrated their occurrence in PE of some marine cyanobacteria (Wilbanks and Glazer 1993, Swanson et al. 1991).

The phycobilisome structure and the phycobiliprotein composition are highly variable in the course of acclimation to varying light intensities, light qualities and nutrient availability (Gantt 1990, Reuter and Müller 1993, Grossman et al. 1994, Talarico 1996). In previous studies, we have demonstrated that the phycobilisomes of the endemic and one of the most common Antarctic red macroalga *P. decipiens* possess a broad range of variability. We reported about the unexpected finding of two hemiellipsoidal phycobilisome forms *P. decipiens* (Lüder et al. 2001b), and their alteration in number and size during acclimation

to seasonally changing light conditions (Lüder et al. 2001a, Lüder et al. 2002b). The phycobilisome size was modulated by changing the PE part within the rods, while the number of the rods remained unaltered. The rod length seems to be altered primarily by coupling and uncoupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. Moreover, we have identified in *P. decipiens* three coloured γ-subunits ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) and three associated PE-subunit complexes ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$) with different absorption characteristics around 544.5 nm and with a red shift in their absorption maxima. Furthermore, a red shift in their fluorescence emission maxima was detected, which probably improve the energy transfer downhill the rod (Lüder et al 2001b). Possible rods and phycobilisome arrangements could be presented in a model (Lüder et al. 2002b).

The present study aimed to investigate the influence of sea ice cover in winter on the phycobilisome assembly in *P. decipiens*. If the ice is covered with snow, dim light or complete darkness prevail in the sublittoral for up to ten months of the year (Zielinski 1990, Miller and Pearse 1991, Drew and Hastings 1992, Klöser et al. 1993). After break up of sea ice in Antarctic spring, the water is very clear, the sunlight penetrates deeply into the water column and the daylength increase rapidly (Klöser et al. 1993). In summer, the water becomes turbid as a result of plankton blooms and glacial melt water (Klöser et al. 1993). How do the algae acclimate to this long period of darkness and to the sudden increase in irradiance in spring?

Since in the Antarctic, sublittoral algae are exposed to almost constant low water temperatures (-1.8 to +2.0 °C) and high nutrient concentrations over the entire year; their seasonal development depends mainly on the strong seasonal variation of light conditions, especially of the daylength (Wiencke 1996). Thus, we were able to monitor the seasonal development of Antarctic species in long-term culture experiments by changing the daylength weekly according to the seasonally fluctuating daylengths measured at the collecting site on King George Island, South Shetland Islands, Antarctica (Wiencke 1990a, 1990b; see also Fig. 1). In long-term culture experiments with *P. decipiens*, winter sea-ice cover was simulated by interrupting the modulation of Antarctic daylengths with a period of six months of darkness during the winter months (April-October). The results have shown that even in darkness, *P. decipiens* started to develop new blades in early August, and growth rates were low or even negative during darkness, but maximal in spring after re-illumination (Weykam et al. 1997). Photosynthetic performance and pigment contents were reduced dramatically at the end of the dark period, but recovered to maximal values in spring (Lüder et al. 2002a, Weykam et al. 1997). A time course of re-illumination showed that *P. decipiens* started by 24 h after re-illumination to accumulate

Chl *a* and to photosynthesise. The phycobiliprotein accumulation started after a lag time of about seven days (Lüder et al. 2002a). The utilisation of floridean starch, accumulated in the previous summer, probably allowed survival during this long period of darkness (Weykam et al. 1997). This all together reflect a high potential of acclimation to varying environmental light conditions in *P. decipiens*.

In the present study, we describe the acclimation process of phycobilisome assembly to seasonally changing light conditions and the influence of a long dark period during the winter months, simulating Antarctic winter sea ice cover, in a long-term culture study of *P. decipiens*. This is the first study, in which phycobilisome assembly was studied in an alga cultivated under darkness over a period of a six months. The appearance of both phycobilisome forms was documented, and changes in the ratios of their phycobiliproteins as well as in their polypeptide composition were determined in monthly intervals. Moreover, a time-scale study was conducted in the first four weeks of re-illumination. The results are discussed in relation to the phycobiliprotein tissue content and the photosynthetic performance (Lüder et al. 2002a).

## MATERIAL AND METHODS

### *Algal material and culture conditions*

Tetraspores of *Palmaria decipiens* (Reinsch) Ricker were collected on King George Island (South Shetland Islands, Antarctica, 62°12'S, 58°58'W and the developing male gametophytes were cultivated in the laboratory under seasonally fluctuating daylengths (mimicking field conditions, Fig. 1) at the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany (Wiencke 1988, Wiencke 1990a). According to Lüder et al. (2002a), several young male gametophytes were grown in twenty-four 3 l glass beakers for about two years under seasonally fluctuating daylengths (Fig. 1), under a constant irradiance of 25 µmol photons $m^{-2}$ $s^{-1}$ using daylight fluorescent tubes (L58/W19, Osram, Munich, Germany) at a constant temperature of 0±18 °C in Provasoli enriched North Sea water (Provasoli 1966, 34 ‰, pH 8), aerated with pressurised air. To ensure sufficient nutrient supply, the culture medium was changed every second week.

In the first year a biomass of about 1200 g fresh weight was generated, and the alga was acclimated during this time to seasonally changing light conditions. At the beginning of the second year, in Antarctic March (14 h and 17 min light per day), the experiment was started and thalli were harvested from different beakers at monthly intervals and used for the isolation of phycobilisomes.

From the 2nd April to 1st October (Fig. 1) the algae were directly exposed to darkness to simulate winter sea-ice cover. Afterwards, they were directly re-illuminated according to the daylength that the species would experience in the field (2nd October: 13h light per day).

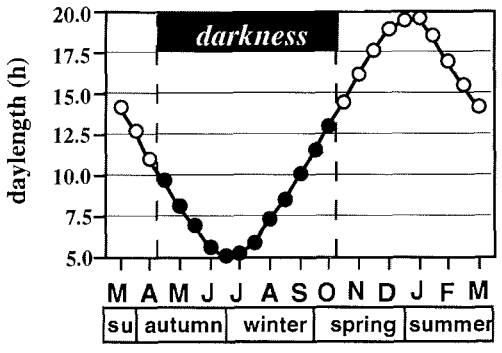


**Fig. 1.** Simulation of seasonal changes of daylength on King George Island, Antarctica, including simulation of winter sea-ice covering by exposure of *P. decipiens* for six months to darkness (2nd April to 1st October). Summer/autumn change: 21. March, autumn/winter change: 21. June, winter/spring change: 21. September, spring/summer change: 21. December.

### *Isolation of phycobilisomes*

The phycobilisomes were isolated as previously described in Lüder et al. (2001b), according to Nies and Wehrmeyer (1980) and Reuter and Wehrmeyer (1990). Each month, approximately the same amount of fresh weight was disrupted by grinding and incubated in 1 % (w/v) N,N-dimethyl-dodecylamine-N-oxide (LDAO, Fluka, Buchs, Switzerland), deoxyribonuclease I (DNase I, 80 mg $ml^{-1}$ extract) and 15 % (w/v) sucrose in 1.5 M potassium phosphate buffer, pH 6.8, for 30 min in the dark at room temperature with gentle stirring. To prevent proteolytic degradation, all buffers contained 1 mM Pefabloc SC (Boehringer, Mannheim, Germany) and 2 mM EDTA. Cell debris was removed by centrifugation for 30 min at 48000 g (Sorvall RC-5B, SS34, Du Pont de Nemours, Bad Homburg, Germany). The supernatant was layered onto a step sucrose gradient of 15, 25, 35 and 45 % (w/v) sucrose in 1.5 M potassium phosphate buffer, pH 6.8, and was ultracentrifuged at 280000 g and 13 °C for 22 h (L5-65, SW 40 Ti, Beckman, Fullerton, CA, USA). After centrifugation the phycobilisomes were collected

with a syringe. The remaining phycobilisomes were pelleted by ultracentrifugation at 280000 g for 3 h (L5-65, Ti 50, Beckman), resuspended in 15 % (w/v) sucrose with 1 mM Pefabloc SC, frozen at -30 °C in aliquots, and used for subsequent electrophoresis.

### *SDS-PAGE*

The polypeptide composition of the phycobilisomes was analysed on 15 % (w/v) polyacrylamide gels as previously described in Lüder et al. (2001b), according to Reuter and Wehrmeyer (1988). For a better resolution of the linker polypeptides 6 % (v/v) isopropanol was added to the separation gel as described by Reuter and Nickel-Reuter (1993). Electrophoresis was performed on ice in a Mini Protean II cell (Bio-Rad) for 2 h at 200 V. Samples containing 10 µg total protein each, were solubilized at 80°C for 10 min in a buffer containing 5 % (w/v) SDS, 2 % (w/v) dithiothreitol, 20 % (w/v) sucrose, 0.25 M Tris HCl, pH 6.8 and 0.02 % (w/v) Coomassie Brillant Blue G250. After electrophoresis, gels were fixed for 1 h in 7 % (v/v) acetic acid with 40 % methanol, stained overnight in 0.025 % (w/v) Coomassie Brillant Blue R250 in 7 % (v/v) acetic acid and destained in 7 % (v/v) acetic acid.

The apparent molecular masses of polypeptides were calculated by using a broad range protein marker SDS-PAGE standard (Bio-Rad). The stained gels were scanned at 520-570 nm with a GS-700 Imaging Densitometer (Bio-Rad) with Multi-Analyst Software (Bio-Rad). The apparent molecular masses of all separated polypeptides were determined and their percentage distributions (% of total PBS protein, stainable with Coomassie blue) were calculated for each month.

### *Phycobiliprotein and protein determination*

The isolated phycobilisomes were dissociated in 0.1 M potassium phosphate buffer, pH 6.8, and the phycobiliprotein content in six different samples was determined by using the equations: PE (µg/ml) = 123.5 * $OD_{565}$ - 73.5 * $OD_{615}$ - 16.3 * $OD_{650}$, PC (µg/ml) = 163.2 * $OD_{615}$ - 117.1 * $OD_{650}$ and APC (µg/ml) = 165.6 * $OD_{650}$ - 16.4 * $OD_{615}$ after Rosenberg (1981).

The protein contents of the isolated PBS were determined with a Bio-Rad-Protein Assay (Bio-Rad, Munich, Germany), according to the instructions of the manufacturer.

## RESULTS

### *Intact phycobilisomes*

Intact undissociated phycobilisomes were isolated in the 35 % (w/v) sucrose layer as one or two discrete bands during the entire year (Fig. 2). Before and after darkness the phycobilisomes appeared in two discrete bands with variation in the appearance of the upper and lower banded phycobilisomes ($PBS_{up}$, $PBS_{low}$). After one month of darkness, two phycobilisome forms were still present, and $PBS_{up}$ dominated. From the second month of darkness on, $PBS_{low}$ disappeared completely, and during continuous darkness the amount of the remaining $PBS_{up}$ decreased strongly. After re-exposure to light, $PBS_{low}$ appeared again in low amounts. In January $PBS_{up}$ and $PBS_{low}$ appeared in equal high amounts. However, in February, $PBS_{low}$ was almost negligible, recovered slightly in March and clearly increased in April.

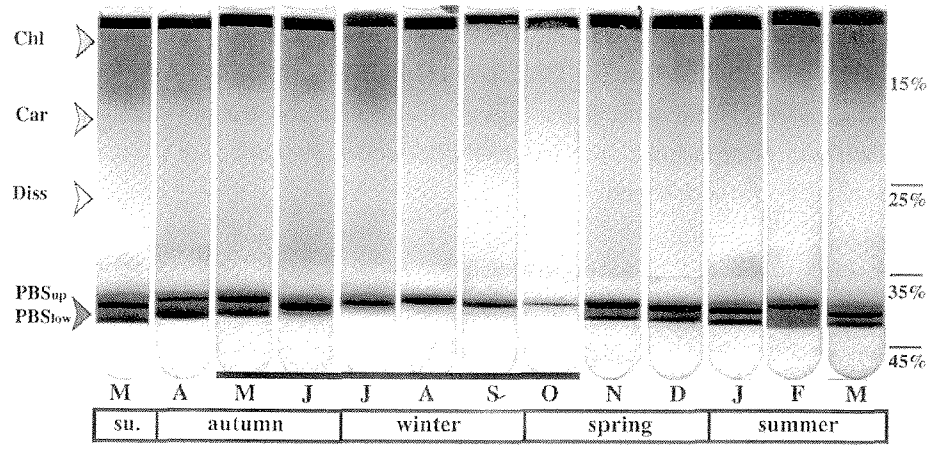


**Fig. 2.** Seasonal changes in the appearance of $PBS_{up}$ and $PBS_{low}$, isolated from *P. decipiens* in step sucrose density gradients of 15, 25, 35 and 45 % (w/v) sucrose. $PBS_{up}$ and $PBS_{low}$, intact phycobilisomes; diss, dissociated phycobilisomes; chl, chlorophyll; car, carotenoids.

Dissociated phycobilisomes were found between the 15 % and 35 % (w/v) sucrose layers. The highest amount was observed between May and July. In the last two months of darkness nearly no dissociated phycobilisomes were present. Free carotenoids and chlorophyll appeared in the 15 % (w/v) sucrose layer. A small dark green layer of membrane bound Chl and cell debris was found on the top of the gradients.

**Phycobiliprotein ratios**

The phycobiliprotein contents of the isolated phycobilisomes were determined and the seasonal changes in their ratios are shown in Fig. 3. In $PBS_{up}$ (Fig. 3A), the ratios of PC:APC were nearly constant around 1.5 before and during the first five months of darkness, indicating no alteration in the number of rods. In the last month of darkness, the decreased in the PC:APC ratio to 0.6, indicating a great loss in total rods. After one month of re-illumination, the rod number was nearly fully recovered.

In the first five months of darkness, the PE:APC and the PE:PC ratios were more or less constant, excluding a sudden rise in August, indicating more or less no alteration in the rod length. In the last month of darkness, a drastic decrease to 6.6 in the PE:APC ratio and a drastic increase to 11.0 in the PE:PC ratio was found, indicating rods mainy containing PE remained intact. After re-illumination the PE:APC ratio increased initially dramatically and then slowly to its highest value of 13.8 in February. In the mean time, the PE:PC ratio decreased until December to 8.8. However, after one month of re-illumination the phycobilisome size was recovered totally, and a further coupling of PE occurred.

In $PBS_{low}$ (Fig. 3B) the ratios of PC:APC, PE:APC and PE:PC remained almost unchanged after one month of darkness, indicating no alteration in the phycobilisome size. From the second month of darkness onwards $PBS_{low}$ disappeared, but occurred again after re-illumination. One month after re-illumination in $PBS_{low}$, the phycobiliprotein ratios reached only values, which are similar to the once found in the last month of darkness in $PBS_{up}$ (PE:APC= 6.7; PE:PC= 11.5; PC:APC= 0.6). The PC:APC ratio was not able to recover totally and achieved maximal 1.3 in February. The PE:APC ratio increased drastically to 13.5 in December, indicating a coupling of rods mainly consistent of PE. The PE:PC ratio increased drastically until January to its highest value (14.3) and decreased in February/March to 10.4. However, after re-illumination the phycobilisome size was not able to recover totally. Instead, the phycoblisome size increased drastically in December by coupling rods mainly consistent of PE (without PC).

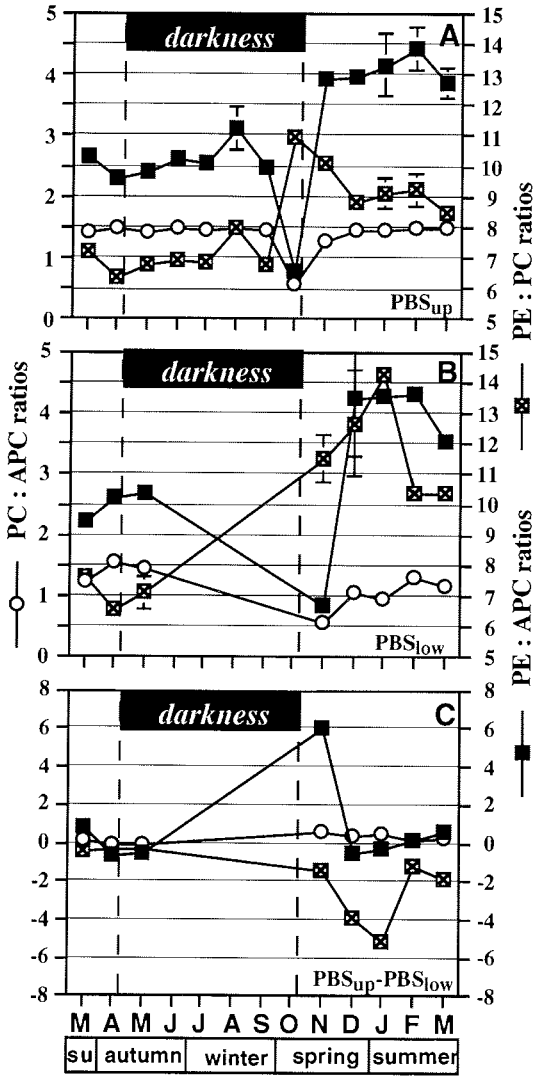


**Fig. 3.** Seasonal changes in the ratios of phycobiliproteins in the phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*. Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value. APC, allophycocyanin; PC, phycocyanin; PE, phycoerythrin.

In Fig. 3 C the differences in the ratios of phycobiliproteins from $PBS_{up}$ and $PBS_{low}$ are shown. Before and during darkness, there was no difference in the phycobiliprotein ratios of $PBS_{up}$ and $PBS_{low}$. After re-illumination the PC:APC and PE:APC ratios, excluding the November value, were also very similar; but the PE:PC ratios were much higher in $PBS_{low}$ than in $PBS_{up}$.

**Linker polypeptides of the rods ($\gamma$-subunits, $L_R$ and $L_{RC}$)**

The seasonal changes of the linker polypeptides, described as percentage ratio of total phycobilisome protein (% of total PBS protein, stainable with Coomassie blue), are presented in Fig. 4. The distribution of linker polypeptides belonging to the rods ($\gamma$-subunits ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$), rod linker ($L_R^{38.9PC}$) and rod-core linker ($L_{RC}^{30.6PC}$)) from $PBS_{up}$ and $PBS_{low}$ are summarised in Fig. 4A and 4D. Both in $PBS_{up}$ and $PBS_{low}$ the $\gamma^{37.3}$-subunit has the highest protein part within all linker polypeptides and made up 4.7 to 6.4 % of total PBS protein, followed by the $\gamma^{40.3}$-subunit with 1.7-3.0 %, the $L_{RC}^{30.6PC}$ with 1.2-2.6 %, and the $L_R^{38.9PC}$ with 1.2-2.1 %. The $\gamma^{33.5}$-subunit showed the greatest changes with 1.4-4.0 % of total PBS protein during the entire year in $PBS_{up}$ and $PBS_{low}$.

To exclude the influence of contaminants, and since the standard deviations of some samples were rather high, we calculated the percentage ratios (%-ratios) within the linker polypeptides belonging to the rod ($\gamma$-subunits, $L_R$ and $L_{RC}$) (Fig. 5). In $PBS_{up}$ the ratio of the $\gamma^{33.5}$-subunit changed very little, between 10.6-12.6 % before and during darkness. The ratios of the remaining linkers ($L_{RC}^{30.6PC}$, $L_R^{38.9PC}$, $\gamma^{37.3}$- and $\gamma^{40.3}$-subunits) were also more or less unchanged before and during the first four months of darkness, indicating no alteration in the phycobilisome size of $PBS_{up}$ and $PBS_{low}$. The $L_{RC}^{30.6PC}$ ranged between 16.8-18.3 % and decreased to nearly the halved value of 8.7 % in the last two month of darkness. The $L_R^{38.9PC}$ showed an almost constant ratio of 13% and decrease to 9.4 % in the last month of darkness. The $\gamma^{40.3}$-subunit ranged between 19.5-21.7 % and fell clearly to 14.8 % in the last month of darkness. In contrast, the $\gamma^{37.3}$-subunit varied between 36-40 % and increased to 48-53% in the last two months of darkness. However, the strong decrease in the amount of $L_{RC}^{30.6PC}$ after five months, and of $L_R^{38.9PC}$ and $\gamma^{40.3}$-subunit after six months of darkness, due to the strong increase in the ratio of the remaining $\gamma^{37.3}$-subunit in the last two months of darkness, indicating the loss of some rods containing PC and PE in $PBS_{up}$. Even in $PBS_{low}$, the %-ratios of the linkers remained unchanged before and during darkness, indicating no alteration in the phycobilisome size. From the second month of darkness onwards, the $PBS_{low}$ disappeared and appeared after re-illumination.

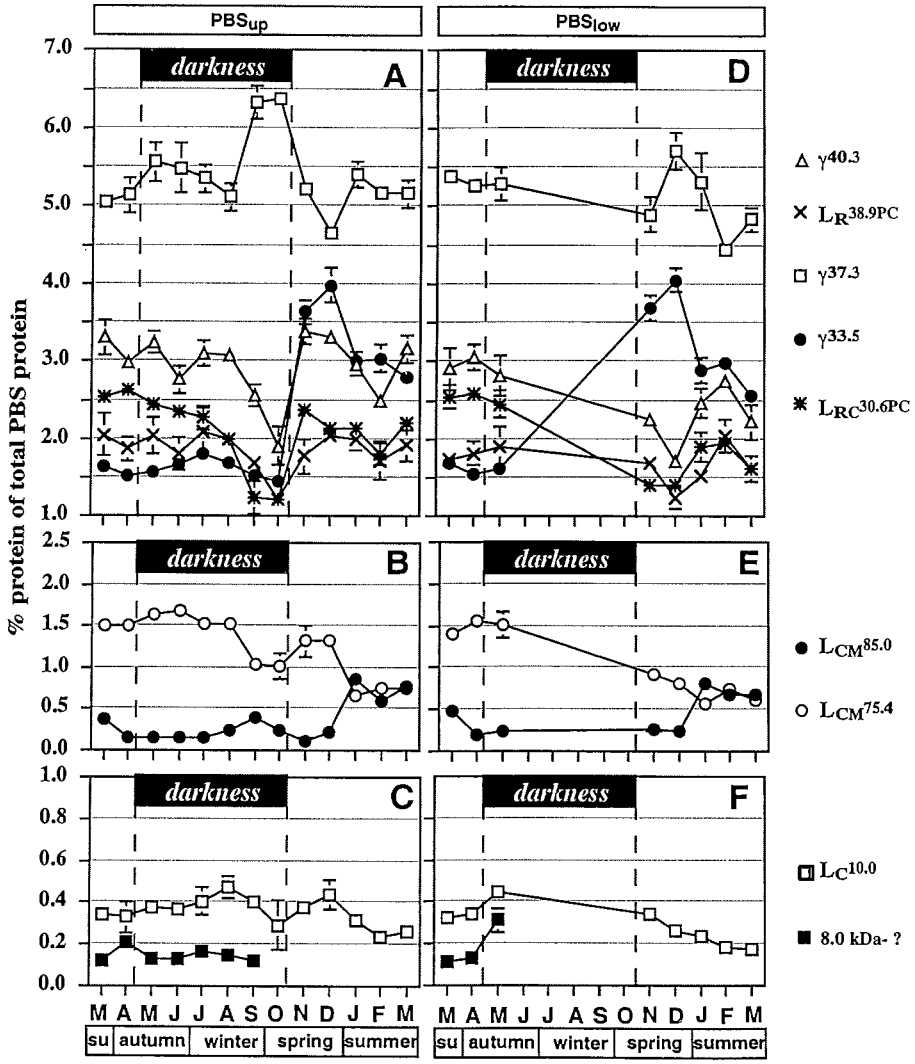


**Fig. 4.** Seasonal changes in the linker polypeptide composition of phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*. Represented as percentage ratio of total phycobilisome protein (% of total PBS protein), densitometrically calculated from the area under peaks of Coomassie blue stained SDS gel scans. Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value. $\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$, γ-subunits; $L_C$, core linker; $L_{CM}$, core membrane linker; $L_R^{PC}$, rod linker associated to phycocyanin;. $L_{RC}^{PC}$, rod-core linker associated to phycocyanin.

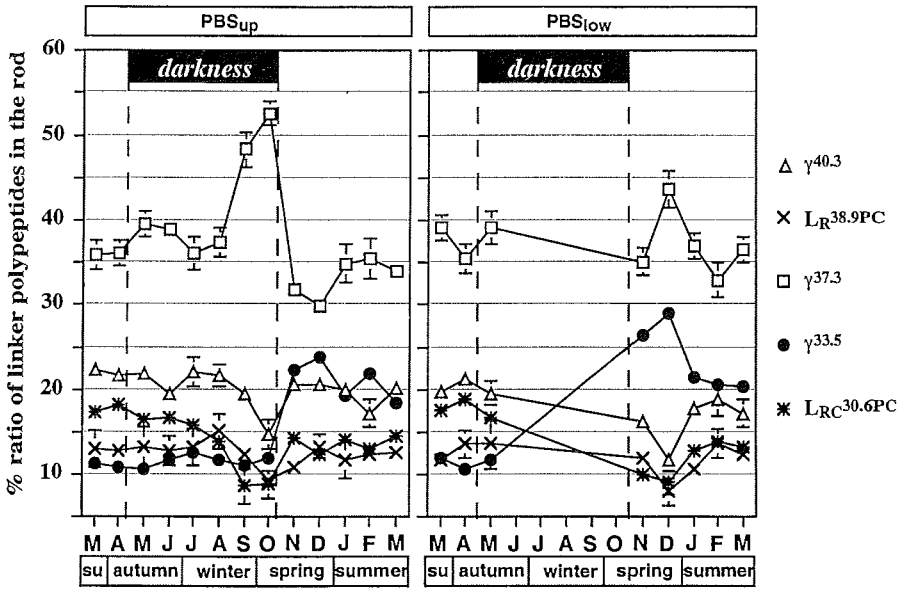


**Fig. 5.** Seasonal changes in the ratios of linker polypeptides belonging to the rods of phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*, and represented as percentage ratio (%-ratio). Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value. $\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$, γ-subunits; $L_R^{PC}$, rod linker associated to phycocyanin; $L_{RC}^{PC}$, rod-core linker associated to phycocyanin.

After one month of re-illumination in $PBS_{up}$, the proportions of all linker polypeptides were fully recovered. Moreover, the amount of the $\gamma^{33.5}$-subunit increased drastically, and a double ratio of 23.9 % was achieved in December, indicating the coupling of further PE. In $PBS_{low}$, the amount of the $\gamma^{33.5}$-subunit increased also drastically after re-illumination, and nearly a threefold ratio of 29 % was achieved in December. Even the $\gamma^{37.3}$-subunit reached its highest ratio of 44% in December. In contrast, the ratios of $L_{RC}^{30.6PC}$ and $\gamma^{40.3}$-subunit were not fully recovered and were lowest. However, in the Antarctic summer months (January-March), the ratios of $L_{RC}^{30.6PC}$, $L_R^{38.9PC}$, the $\gamma^{37.3}$- and the $\gamma^{40.3}$-subunit were similar to the values before darkness. The amount of the $\gamma^{33.5}$-subunit strongly decreased, but the ratios were still doubled compare to the values before

darkness, both in $PBS_{up}$ and $PBS_{low}$, indicating a reduction in phycobilisome size by uncoupling of some PE.

**Linker polypeptides of the core ($L_{CM}$ and $L_C$).**

In Fig. 4B and 4E, the seasonal changes of the large core membrane linkers ($L_{CM}^{75.4}$ and $L_{CM}^{85.0}$), expressed as % of total PBS protein, are shown for $PBS_{up}$ and $PBS_{low}$.

Before and during the first four months of darkness, the $L_{CM}^{75.4}$ comprised about 1.5 % of total PBS protein and the $L_{CM}^{85.0}$ about 0.3 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$. In the last two months of darkness in $PBS_{up}$, the $L_{CM}^{75.4}$ clearly decreased and the $L_{CM}^{85.0}$ slightly increased in their ratios. After re-illumination from January on, equal amounts of $L_{CM}^{75.4}$ and $L_{CM}^{85.0}$ were found in $PBS_{up}$ and $PBS_{low}$.

The seasonal changes in the small core linker $L_C^{10.0}$ and in the 8.0 kDa polypeptide expressed as % of total PBS protein are shown in Fig. 4 C and F for $PBS_{up}$ and $PBS_{low}$. $L_C^{10}$ made up about 0.2-0.5 % of total PBS protein, remaining more or less constant during the entire year, both in $PBS_{up}$ and $PBS_{low}$. The 8.0 kDa polypeptide was found in the first part of the year, between March and September, and made up about 0.1-0.3 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$.

**Contaminants.**

The seasonal changes of the polypeptides thought to be contaminants (expressed as % of total PBS protein stainable with Coomassie) are summarised in Fig. 6. The 65.3 kDa and 53.3 kDa polypeptides occurred over the whole year, ranging between 0.1-0.6 % and 0.3-0.7 % of total PBS protein, in $PBS_{up}$ and $PBS_{low}$ respectively. The proportion of the 53.3 kDa polypeptide increased after re-illumination, in November in $PBS_{up}$ and in February in $PBS_{low}$. The 50.2 kDa polypeptide was found before and during darkness and after re-illumination, but disappeared from December on in $PBS_{low}$ and from January on in $PBS_{up}$. The 60.7 kDa, 56.6 kDa and 47.9 kDa polypeptides appeared before and during darkness in $PBS_{up}$ and $PBS_{low}$. The 57.9 kDa and 52.0 kDa polypeptides were found during darkness and after re-illumination, both in $PBS_{up}$ and $PBS_{low}$. In $PBS_{up}$, a drastical increase in their ratio was observed in the last month of darkness and a drastic fall was seen during re-illumination.

## DISCUSSION 'in preparation'

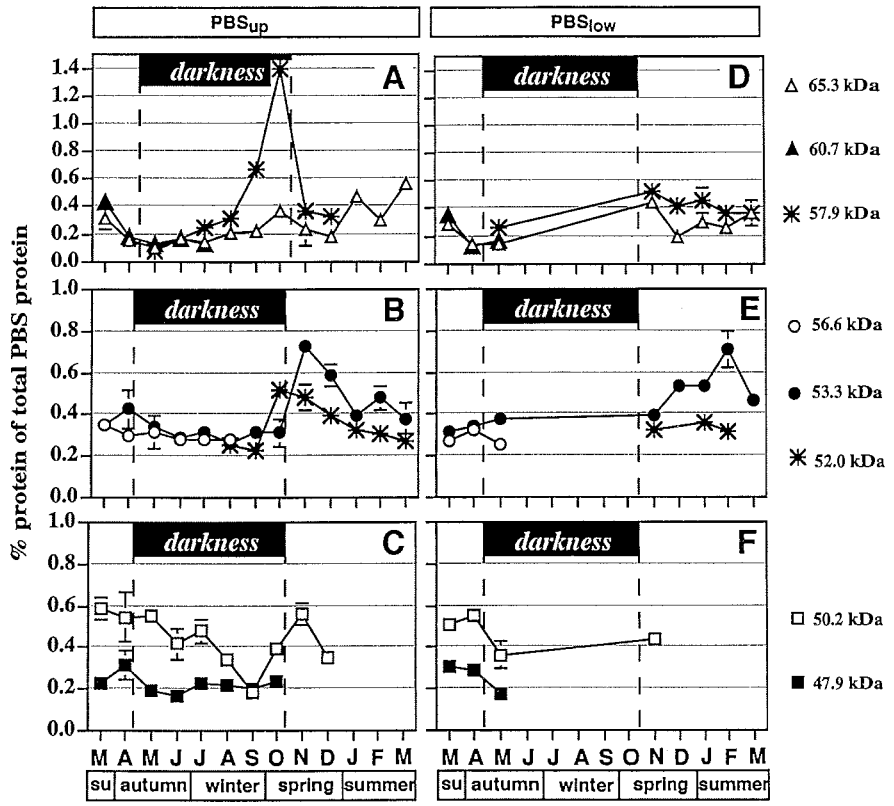


**Fig. 6.** Seasonal changes in the composition of polypeptides, thought to be contaminants or associated to the phycobilisomes ($PBS_{up}$ and $PBS_{low}$) isolated from *P. decipiens*. Represented as percentage ratio of total phycobilisome protein (% of total PBS protein), densitometrically calculated from the area under peaks of Coomassie blue stained SDS gel scans. Means and SD, n= 6; in those cases where no error bars are given, the SD is smaller than the symbol for the mean value.

## ACKNOWLEDGEMENT

This work was supported by grants from the Deutsche Forschungsgemeinschaft. We gratefully thank Erika Lorenz and Ute Hemboldt for excellent technical support. We acknowledge J. Marquard for helpful comments.

## REFERENCES

Bald D, Kruip J, Rögner M (1996) Supramolecular architecture of cyanobacterial thylakoid membranes: How is the phycobilisome connected with the photosystems ? Photosynth Res 49:103-118

Drew, E. A. & Hastings, R. M. 1992. A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. *Phycologia* 31:262-277.

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of the Red Algae. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney, pp 203-219

Glazer AN (1985) Light harvesting by Phycobilisomes. Annu Rev Biophys Biophys Chem 14:47-77

Glazer AN, Hixson CS (1977) Subunit structure and chromophore composition of rhodophytan phycoerythrins *Porphyridium cruentum* B-phycoerythrin and b-phycoerythrin. J Biol Chem 252:32-42

Grossman AR, Schaefer MR, Chiang GG, Colier JL (1994) The responses of cyanobacteria to environmental conditions: light and nutrients. In: Bryant DA (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Netherlands, pp 641-675

Klöser, H., Ferreyra, G., Schloss, I., Mercuri, G., Laturnus, F. & Curtosi, A. 1993. Seasonal variation of algal growth conditions in sheltered Antarctic bays: the example of Potter Cove (King George Island, South Shetlands). *J. mar. Syst.* 4:289-301.

Lüder UH, Knoetzel J, Wiencke C (2001a) Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. Polar Biol 24:598-603

Lüder UH, Knoetzel J, Wiencke C (2001b) Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae). Physiol Plant 112:572-581

Lüder UH, Knoetzel J, Wiencke C (2002a) Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta) - a study to simulate Antarctic winter sea ice cover.J Phycol (submitted and revised).

Lüder UH, Knoetzel J, Wiencke C (2002b) Acclimation of phycobilisome assembly to seasonally changing light conditions in the Antarctic red macroalga *Palmaria decipiens* (Florideophyceae). Planta (prepared to be submitted)

MacColl R (1998) Cyanobacterial phycobilisomes. J Struct Biol 124:311-334

Miller, K. A. & Pearse, J. S. 1991. Ecological studies of seaweeds in McMurdo Sound, Antarctica. *Am. Zool.* 31:35-48.

Mörschel E (1991) The light-harvesting antennae of cyanobacteria and red algae. Photosynthetica 25:137-144

Nies M, Wehrmeyer W (1980) Isolation and biliprotein characterization of phycobilisomes from the thermophilic Cyanobacterium *Mastigocladus laminosus*. Planta 150:330-337

Provasoli L (1966) Media and prospects for the cultivation of marine algae. Proc Jpn Conf Hakone 63-75

Reuter W, Müller C (1993) Adaptation of the photosynthetic apparatus of cyanobacteria to light and $CO_2$. J Photochem Photobiol B:Biol 21:3-27

Reuter W, Nickel-Reuter C (1993) Molecular assembly of the phycobilisomes from the cyanobacterium *Mastigocladus laminosus*. J Photochem Photobiol B:Biol 18:51-66

Reuter W, Wehrmeyer W (1988) Core substructure in *Mastigocladus laminosus* phycobilisomes. I. Microheterogeneity in two of three allophycocyanin core complexes. Arch Microbiol 150:534-540

Reuter W, Wehrmeyer W (1990) Core substructure in *Mastigocladus laminosus* phycobilisomes. II. The central part of the tricylindrical core - $AP_{CM}$ - contains the "anchor" polypeptide and no allophycocyanin B. Arch Microbiol 153:111-117

Rosenberg G (1981) Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp (Chlorophyceae). Ph D thesis, Yale University, New Haven, Connecticut

Sidler WA (1994) Phycobilisome and phycobiliprotein structures In: Bryant, D A (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Netherlands, pp 139-216

Stadnichuk IN (1995) Phycobiliproteins: determination of chromophore composition and content. Phytochemical Analysis 6:281-288

Swanson RV, Ong LJ, Wilbanks SM, Glazer AN (1991) Phycoerythrins of marine unicellular cyanobacteria. II. Characterization of phycobiliproteins with unusually high phycourobilin content. J Biol Chem 266:9528-9534

Talarico L (1996) Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. Sci Mar 60:205-222

van Thor J J, Mullineaux CW, Matthijs HCP, Hellingwerf KJ (1998) Light harvesting and state transitions in cyanobacteria. Bot Acta 111:430-443

Weykam, G., Thomas, D. N. & Wiencke, C. 1997. Growth and photosynthesis of the Antarctic red algae *Palmaria decipiens* (Palmariales) and *Iridaea cordata* (Gigartinales) during and following extended periods of darkness. *Phycologia* 36:395-405.

Wiencke C (1988) Notes on the development of some benthic marine macroalgae of King George Island, Antarctica. Ser Cient INACH 37:23-47

Wiencke C (1990a) Seasonality of brown macroalgae from Antarctica - a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:589-600

Wiencke C (1990b) Seasonality of red and green macroalgae from Antarctica - a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601-607

Wiencke C (1996) Recent advanes in the investigation of Antarctic macroalgae. Polar Biol 16:231-240

Wilbanks SM, Glazer AN (1993) Rod structure of a phycoerythrin II-containing phycobilisome. II. Complete sequence and bilin attachment site of a phycoerythrin γ-subunit. J Biol Chem 268:1236-1241

Zielinski, K. 1990. Bottom macroalgae of the Admiral Bay (King George Island, South Shetlands, Antarctica). *Pol. Polar Res.* 11:95-131.

# 4. SUMMARY OF RESULTS

## 4.1. Isolation and characterisation of phycobilisomes

***Double-banded phycobilisomes ($PBS_{up}$ and $PBS_{low}$)***

Phycobilisomes from *Palmaria decipiens* were isolated on discontinuous sucrose gradients in the 35 % (w/v) sucrose layer in two discrete bands, and not in one as expected. To exclude methodical faults, we also isolated phycobilisomes from the temperate *Palmaria palmata* and hemiellipsoidal phycobilisomes from the unicellular red alga *Porphyridium cruentum* and hemidiscoidal phycobilisomes from *Rhodella violacea*. In *P. palmata* the phycobilisomes were also separated in two discrete bands, whereas the phycobilisomes from *Porphyridium* and *Rhodella* were found in one band (Publ. 1, Fig. 1). The double banded phycobilisomes ($PBS_{up}$ and $PBS_{low}$) from *P. decipiens* were further characterised.

***Intactness of $PBS_{up}$ and $PBS_{low}$***

The intactness of $PBS_{up}$ and $PBS_{low}$ was proven by low and room temperature fluorescence emission spectroscopy. At 77 K, the emission peaks of $PBS_{up}$ and $PBS_{low}$ were at 680 nm, which indicates an effective energy transfer to the terminal energy acceptors (core membrane linker $L_{CM}$ and APC-B) of the phycobilisome (Publ. 1, Fig. 2A).

***Absorption and fluorescence characteristics of $PBS_{up}$ and $PBS_{low}$***

No evidence for qualitative and quantitative differences between $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* was found in their absorption spectra (Publ. 1, Fig. 3) and in their phycobiliprotein ratios (PE:APC, PC:APC and PE:PC; Publ. 1, Table 1).

The absorption spectra (Publ. 1, Fig. 3 and Fig. 5) allowed us to identify the RIII-phycoerythrin type (RIII-PE: 496 < 544 < 567 nm), RI-phycocyanin (RI-PC: 555 < 617 nm) and allophycocyanin (APC: 652 nm), both in $PBS_{up}$ and $PBS_{low}$, according to Glazer and Hixon (1975), Rennis and Ford (1992), Ducret et al. (1994) and Sidler (1994).

***Polypeptide composition of $PBS_{up}$ and $PBS_{low}$***

The polypeptide composition of $PBS_{up}$ and $PBS_{low}$ was separated by SDS-PAGE (lane two in Publ. 1, Fig.7) and the individual polypeptides were identified (Publ. 1, Table 3).

$PBS_{up}$ and $PBS_{low}$ had α- and β-subunits and linker polypeptides of the same molecular mass. The apparent molecular masses of the phycobiliprotein subunits (α and β) ranged from 16.8 to 21.6 kDa. Three γ-subunits with molecular masses of 33.5 kDa, 37.3 kDa and 40.3 kDa ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) were identified. Colourless linker polypeptides of 38.9 kDa, probably the PC rod linker ($L_R^{38.9PC}$), and of 30.6 kDa, probably the PC-APC rod-core linker ($L_{RC}^{30.6PC}$) were found. Furthermore a small core linker of 10 kDa ($L_C^{10.0}$) and a large core membrane linker of 85.0 kDa ($L_{CM}^{85.0}$) were present. In *P. decipiens,* the phycobiliprotein subunits (α and β) accounted for approximately 80 % and the linker polypeptides (incl. γ-subunits) for about 17 % of the total proteins stainable with Coomassie blue.

The percentage distribution of the subunits and of the linker polypeptides in $PBS_{up}$ and $PBS_{low}$ was similar, except that the amount of the small core linker $L_C$, the large anchor linker $L_{CM}$ and the 40.3 kDa γ-subunit were minimally higher in $PBS_{up}$.

The remaining polypeptides were often found in SDS gels of phycobilisomes. Polypeptides of approximately 45-50 kDa were supposed to be FNR (ferredoxin:$NADP^+$ oxidoreductase), which is associated with the phycobilisomes, probably attached to the peripheral rods (Schluchter and Bryant 1992, Sidler 1994, Ritz et al.1998, see Introduction, Fig. 2). Proteolytic enzymes of 50-66 kDa, which are involved in the degradation of the linker polypeptides, were found even in highly purified phycobiliprotein complexes of phycobilisomes (Reuter and Müller 1993). A 54 kDa polypeptide was identified as the large subunit of Rubisco and was interpreted to be a contaminant (Marquardt et al. 1999).

### *Trimeric and hexameric subunit complexes in $PBS_{up}$ and $PBS_{low}$*

The phycobiliprotein subunit complexes of $PBS_{up}$ and $PBS_{low}$ were separated by native PAGE into one APC-subunit complex (6-APC), one PC-subunit complex (2-PC), and four PE-subunit complexes (1-PE , 3-PE, 4-PE and 5-PE) (Publ. 1, Fig. 4). By use of the absorption (Publ. 1, Fig. 5) and fluorescence emission (Publ. 1, Fig. 6) characteristics, and of the polypeptide composition analysed by SDS-PAGE (Publ. 1, Fig. 7), the phycobiliprotein subunits and some linker polypeptides from $PBS_{up}$ and $PBS_{low}$ (mentioned above) were identified and trimeric and hexameric subunit complexes were reconstructed (summarised in Publ. 1, Table 2).

The most interesting result was, that in $PBS_{up}$ and $PBS_{low}$, the three γ-subunits formed three hexameric PE-subunit complexes (3-PE: $(\alpha\beta)_6\gamma^{33.5}$, 4-PE: $(\alpha\beta)_6\gamma^{37.3}$, 5-PE: $(\alpha\beta)_6\gamma^{40.3}$), with different absorption characteristics around 544 nm and with a red shift in their absorption maxima, both in $PBS_{up}$ and $PBS_{low}$. Moreover, a red shift in their

fluorescence emission maxima was detected. A second lower fluorescence emission maximum at 595nm was found in the $(\alpha\beta)_6\gamma^{33}$ subunit complex.
The remaining 1-PE-subunit complex was suspected to be r-phycoerythrin, forming trimeric subunit complexes ($(\alpha\beta)_3$ or $(\alpha\beta)_3\gamma^{33.5}$), showing a very low fluorescence signal (compared to the others PE complexes; Publ. 1, Fig. 6), but made up about 21 % of the total PE content, both in $PBS_{up}$ and $PBS_{low}$. The 5-PE subunit complex made up the largest proportion, with 42 % of the total PE content, followed by the 3-PE (29 %), 1-PE (21 %) and 4-PE complex (8 %).
However, $PBS_{up}$ and $PBS_{low}$ differed only in the 1-PE subunit complex, suspected to be r-phycoerythrin. The 1-PE complex of $PBS_{low}$ showed drastically reduced absorbances at 534 nm and at 565 nm (Publ. 1, Fig. 5), in spite of presence of all three PE-subunits ($\alpha$, $\beta$, and $\gamma$) was identified by SDS-PAGE (Publ. 1, Fig. 7). Conversely, the 1-PE complex of $PBS_{up}$ showed a "normal" absorption spectrum, but in the SDS-PAGE no $\gamma$-subunit was found and three low molecular weight polypeptides appeared, probably dissociation products of the missing $\gamma$-subunit. We assumed a weaker stability of this $\gamma$-subunit, which is important for the incorporation of PE in the phycobilisome, in the 1-PE complex of the $PBS_{up}$, relative to $PBS_{low}$.

***Negative staining of $PBS_{up}$ and $PBS_{low}$***

The electron micrographs showed negatively stained $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* of the same hemiellipsoidal phycobilisome type with pure structural details (Publ. 1, Fig. 8). The $PBS_{up}$ had a basal length of about 57-64 nm and a height of about 35 nm. The $PBS_{low}$ seemed to be somewhat smaller with a basal length of 50-57 nm and a height of about 32-35 nm.

All results together, suggest that the $PBS_{low}$ is a closer packed and $PBS_{up}$ a little more loosely aggregated hemiellipsiodal phycobilisome form in *P. decipiens*.

## 4.2. Acclimation of photosynthesis, pigments and phycobilisome assembly to seasonally changing daylength

### 4.2.1. Acclimation of photosynthesis and pigments (tissue content)

The optimal quantum yield ($F_v/F_m$) remained constantly high between 0.62 and 0.67 during mid autumn, winter and spring (May-January) but was lower between 0.47 and

0.50 in February and March, increasing again in April (Publ. 2, Fig. 1B). Lowest $ETR_{max}$ values were found in summer, between February and April. $ETR_{max}$ increased more or less continuously from April/May onwards to its highest values in September/October (Publ. 2, Fig. 1B).

Lowest phycobiliprotein tissue content was found in summer, between February and April. (Publ. 2, Fig. 1C). All three phycobiliproteins (APC, PC and PE) started to increase in April/May, more or less in parallel with $ETR_{max}$, but reached maximal values slightly later in October (APC and PC) and in November (PE). A marked decrease in the APC and PC tissue contents were noticed in December, while PE remained unchanged. Tissue content of PE, APC and PC decreased to about the half value in February. The variation of the APC tissue content indicates an alteration of the phycobilisome number.

Chlorophyll *a* (Chl *a*) tissue content started to increase later in July, and was also highest in October/November.

In autumn, winter and early spring (April-October), a positive correlation was found between $ETR_{max}$ and the total phycobiliprotein tissue content (Publ. 2, Fig. 2A); whereas between $ETR_{max}$ and Chl *a* the correlation was weaker (Publ. 2, Fig. 2B).

The seasonal changes in the ratios of total phycobiliproteins ($PB_{tot}$) to Chl *a* ($PB_{tot}$:Chl *a*) were very similar to the changes in APC:Chl *a* (Publ. 2, Fig. 1D). $PB_{tot}$:Chl *a* and APC:Chl *a* ratios were highest in June/July, when the light was minimised to 5h per day, and lowest in summer (February/April). This indicates, an alteration in the stoichiometry of PBS:PSII:PSI.

The ratios of phycobiliproteins are shown in Fig. 1E (Publ. 2). The PE:APC and the PE:PC ratios were lowest in October, indicating a shortening of rod length, and high in May and December/January, indicating an elongation of rod length. The PC:APC ratio was more or less constant, but there might be a slight increase in August/September and in January, indicative of changes in the number of rods. Overall, this makes an alteration of the phycobilisome size probably by changing rod length and rod number most probable.

### 4.2.2. Acclimation of phycobilisome assembly

Under the same culture conditions the appearance of the upper and lower banded phycobilisomes ($PBS_{up}$, $PBS_{low}$) changed clearly during the entire Antarctic year (Publ. 3, Fig. 2). Between February and May one of the two phycobilisome forms was present in high amounts, whereas the other one was found in very low amounts. In contrast,

between June and January both $PBS_{up}$ and $PBS_{low}$ were present in high amounts, but with changing dominance.

***Phycobiliprotein ratios in $PBS_{up}$ and $PBS_{low}$***

Seasonal changes of the PE:APC, PE:PC and PC:APC ratios of $PBS_{up}$ and $PBS_{low}$ are shown in Fig. 3 (Publ. 3). $PBS_{up}$ and $PBS_{low}$ acclimated to the seasonally changing daylength by changing the PE:PC and PE:APC ratios, whereas the PC:APC ratio was nearly constant at 1.5, indicating no alteration in the number of rods. The highest ratios of PE:APC and of PE:PC were reached in Antarctic spring (November) both in $PBS_{up}$ and $PBS_{low}$. The lowest ratios of PE:APC and of PE:PC were found just after Antarctic summer (April), both in $PBS_{up}$ and $PBS_{low}$. Thus, the phycobilisome size was smallest in April and largest in November, in the following called 'small' phycobilisomes and 'large' phycobilisomes.

During the entire year, the PE:APC ratio increased more or less continuously slightly between April and July, both in $PBS_{up}$ and $PBS_{low}$. From August on, a clear continuous increase in the PE:APC and PE:PC ratios until November was found. This points to a more or less continuous increase in phycobilisome size from April until November, both in $PBS_{up}$ and $PBS_{low}$. From December on, when nearly the maximal daylength was reached, both $PBS_{up}$ and $PBS_{low}$ clearly started to decrease in size, and attained lowest sizes in March.

***Linker polypeptides of the rods ($\gamma$-subunits, $L_R$ and $L_{RC}$)***

The polypeptide composition of $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* was analysed by SDS-PAGE. The seasonal changes of the linker polypeptides belonging to the rod ($\gamma$-subunits, $L_R$ and $L_{RC}$) were described as seasonal changes in their percentage ratios (%-ratios) (Publ. 3, Fig. 5). First, a comparison of the 'small' and the 'large' phycobilisomes is made (see also Publ. 3, Table 1). In the 'small' phycobilisomes (April), the %-ratio was more or less maximal for $L_{RC}^{30.6PC}$, $L_R^{38.9PC}$ and for the $\gamma^{40.3}$-subunit, high for the $\gamma^{37.3}$-subunit and minimal for the $\gamma^{33.5}$-subunit. In contrast, in the 'large' phycobilisomes (November), the %-ratio was minimal for $L_{RC}^{30.6PC}$, $L_R^{38.9PC}$ and for the $\gamma^{40.3}$-subunit, and maximal for the $\gamma^{37.3}$- and $\gamma^{33.5}$-subunits. This suggests a location of the $\gamma^{40.3}$-subunit at the innermost, the $\gamma^{37.3}$-subunit in an intermediary position and the $\gamma^{33.5}$-subunit at the periphery of the PE part of the rods.

The seasonal variation of the linker polypeptides from $PBS_{up}$ and $PBS_{low}$ was very similar. In both $PBS_{up}$ and $PBS_{low}$, the $L_{RC}^{30.6PC}$ and the $L_R^{38.9PC}$ did not vary with the seasons, their %-ratios ranged only by about 3%. In contrast, clear seasonal changes

were seen in the $\gamma^{33.5}$-subunit. It showed with about 11 % the lowest ratio of the entire year in April, but increased during autumn, winter and spring to its double ratio of about 20 % in November, both in $PBS_{up}$ and $PBS_{low}$. The opposite was seen for the $\gamma^{40.3}$-subunit. Its highest ratios were found in March/April (22 %/ 21 %), and the lowest in December/ November (19 %/ 17 %) in $PBS_{up}$ and $PBS_{low}$, respectively. However, these changes were so small, that we assume no alteration in the amount of the $\gamma^{40.3}$-subunit during the entire year. The lowest ratios of the predominant $\gamma^{37.3}$-subunit were seen in October (32 % and 33 %) both in $PBS_{up}$ and $PBS_{low}$. But the highest ratios were reached in June (38 %) for $PBS_{up}$ and in November (39 %) for $PBS_{low}$. The reason is, in $PBS_{up}$ and may be also in $PBS_{low}$, the amount of $\gamma^{37.3}$-subunit slightly increased in parallel with the $\gamma^{33.5}$-subunit until June. Then the amount of the $\gamma^{37.3}$-subunit remained unchanged but the amount of the $\gamma^{33.5}$-subunit continued to increase, which caused the decreased in the relative proportion of the $\gamma^{37.3}$-subunit.

***Linker polypeptides of the core ($L_{CM}$ and $L_C$)***

Seasonal changes of the large core membrane linkers ($L_{CM}^{75.4}$ and $L_{CM}^{85.0}$), were expressed as % of total PBS protein and are shown in Publ. 3 (Fig. 4 B and E). Between March and July, the $L_{CM}^{75.4}$ comprises about 1.5 % of total PBS protein and the $L_{CM}^{85.0}$ about 0.3 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$. Between September and January, however, $L_{CM}^{75.4}$ decreased to only 0.3 % and $L_{CM}^{85.0}$ reached 1.5% of total PBS protein. In February and March equal amounts of $L_{CM}^{75.4}$ and $L_{CM}^{85.0}$ were found in $PBS_{up}$ and $PBS_{low}$.

The seasonal changes in the small core Linker ($L_C$) and the 8.0 kDa polypeptide expressed as % of total PBS protein are shown in Fig. 4C and 4F (Publ. 3) for $PBS_{up}$ and $PBS_{low}$. $L_C^{10}$ made up about 0.3-0.4 % of total PBS protein, this amount remained constant during the entire year, both in $PBS_{up}$ and $PBS_{low}$. An unknown 8.0 kDa polypeptide was found in the first part of the year, between March and August, and made up about 0.1-0.2 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$.

***Contaminants***

The seasonal changes of the polypeptides thought to be contaminants (expressed as % of total PBS protein stainable with Coomassie) are summarised in Fig. 6 (Publ. 3) The 65.3 kDa, 60.7 kDa and 53.3 kDa polypeptides were present over the entire year. The 65.3 kDa and 60.7 kDa polypeptides were almost doubled in the second part of the year (from September on), both in $PBS_{up}$ and $PBS_{low}$. The 53.3 kDa polypeptide increased strongly in February and March in $PBS_{low}$, when compared with $PBS_{up}$. The 56.6 kDa polypeptide

was found only in the first part of the year, and the 57.9 kDa polypeptide appeared only in the second part, both in $PBS_{up}$ and $PBS_{low}$. The 50.2 kDa and 47.9 kDa polypeptides were found between March and August, and again in January/February, both in $PBS_{up}$ and $PBS_{low}$.

## 4.3. Acclimation of photosynthesis, pigments and phycobilisome assembly during and after six months of darkness - a study to simulate Antarctic winter sea ice cover

### 4.3.1. Photosynthesis and pigments (tissue content) during darkness and after re-exposure to light

After three months in darkness $ETR_{max}$ and $F_v/F_m$ started to decrease considerably (Publ. 4, Fig. 1B). After six months of darkness *P. decipiens* lost its ability to photosynthesise. However, in November, one month after re-exposure to light, $ETR_{max}$ and $F_v/F_m$ were fully recovered and $ETR_{max}$ increased to the highest values of this experiment. In the following summer months, $ETR_{max}$ decreased again whereas $F_v/F_m$ continued to increase until February.

During darkness, in the first three months a slight increase in the Chl *a*, PE and PC tissue contents was observed (Publ. 4, Fig. 1C). Phycobiliprotein tissue contents started to decrease after four months and the Chl *a* levels after five months of darkness. After six months of darkness the thalli appeared light green to white corresponding to the lowest phycobiliprotein and low Chl *a* concentrations. After re-exposure to light, all pigments increased rapidly and the tissue concentrations of phycobiliproteins were maximal in December; the concentration of Chl *a* was also very high. The phycobiliproteins began to decrease in January, whereas Chl *a* continued to increase. The changes in the APC content indicates changes in the amount of phycobilisomes.

The seasonal changes in the ratios of total phycobiliproteins ($PB_{tot}$) to Chl *a* ($PB_{tot}$:Chl *a*) are very similar to the changes in APC:Chl *a* (Publ. 4, Fig. 1D). Both parameters decreased during darkness to their lowest values and increased after re-exposure to light to their highest values in December. This indicates, an alteration in the stoichiometry of PBS:PSII:PSI.

The ratios of phycobiliproteins are shown in Fig. 1E (Publ. 4). A clear reduction in the PE:APC and PC:APC ratios from the fourth month of darkness onwards, indicates a loss of rods from the phycobilisomes and the start of its degradation. After re-exposure to

light, PC:APC and PE:APC dramatically increased until January.

24h after re-illumination (Publ. 4, Fig. 4) *P. decipiens* started to increase the content of Chl *a* and to photosynthesise. The phycobiliprotein accumulation (may be equal to synthesis) started after a lag time of about seven days. *P. decipiens* reached $ETR_{max}$ values, comparable to the values before darkness, seven days and maximal values after 30 days of re-illumination.

A strong relation between pigment content and photosynthetic performance was evident by reason of a positive correlation between Chl *a* content and $ETR_{max}$ as well as between Chl *a* content and $F_v/F_m$ during re-illumination (Publ. 4, Fig. 5). In contrast, the relationship between the total phycobiliprotein content ($PB_{tot}$) and $ETR_{max}$ as well as between $PB_{tot}$ and $F_v/F_m$ during re-illumination of *P. decipiens* followed a logarithmic form, and a positive correlation between $PB_{tot}$ content and $ETR_{max}$ as well as between $PB_{tot}$ content and $F_v/F_m$ could be identified only at low $PB_{tot}$ contents (below about 250 $\mu g \cdot g^{-1}$ FW). Even the regression analysis over the entire year showed, that $ETR_{max}$ increased in two steps proportional to the $PB_{tot}$ content: At lower $PB_{tot}$ contents the positive effect of $ETR_{max}$ is much higher than at higher $PB_{tot}$ contents (Publ. 4, Fig. 2)

At last, seasonal changes in the percent ratio of dry to fresh weight (DW:FW) are shown in Fig. 3 (Publ. 4). During darkness, the DW:FW ratio was rather similar around 11.5 %, but a marked peak in August was noticed. After re-exposure to light the DW:FW ratio increased to its highest value of 16.6 % in December.

### 4.3.2. Phycobilisome assembly during darkness and after re-exposure to light

After two month of darkness, the $PBS_{low}$ disappeared completely, and during continuous darkness the amount of the remaining $PBS_{up}$ decreased strongly (Publ. 5, Fig. 2). After re-exposure to light, the $PBS_{low}$ appeared again in low amounts. In January $PBS_{up}$ and $PBS_{low}$ appeared in equal high amounts. However, in February $PBS_{low}$ was almost negligible again.

***Phycobiliprotein ratios in $PBS_{up}$ and $PBS_{low}$***

During the first five months of darkness in $PBS_{up}$, the PE:APC, PE:PC and PC:APC ratios were more or less unchanged, indicating no changes in phycobilisome size (Publ. 5, Fig. 3). A great loss in rods was identified by a strong decrease in the PC:APC ratio in the last month of darkness. Further, the PE:APC ratio decreased dramatically, whereas

the PE:PC ratio showed the opposite response. This suggests, that rods mainly containing PE remained intact. However, after one month of re-illumination, the phycobilisome size was recovered totally and a further coupling of PE occurred.

Even $PBS_{low}$ did not alter in size in the first month of darkness. After re-illumination, in $PBS_{low}$ the PC:APC ratio was not able to fully recover, and the PE:APC ratio increased first after two months of light.

***Linker polypeptides of the rods ($\gamma$-subunits, $L_R$ and $L_{RC}$)***

The polypeptide composition of $PBS_{up}$ and $PBS_{low}$ from *P. decipiens* was analysed by SDS-PAGE. The seasonal changes of the linker polypeptides belonging to the rods ($\gamma$-subunits, $L_R$ and $L_{RC}$) were described as seasonal changes in their percentage ratios (%-ratios) (Publ. 5, Fig. 5). The proportion of the $\gamma^{33.5}$-subunit changed very little, between 10.6-12.6 % before and during darkness, both in $PBS_{up}$ and $PBS_{low}$. The proportions of the remaining linker ($L_{RC}^{30.6PC}$, $L_R^{38.9PC}$, $\gamma^{37.3}$- and $\gamma^{40.3}$-subunits) were also more or less unchanged before and during the first four months of darkness, indicating no alteration in the phycobilisome size of $PBS_{up}$ and $PBS_{low}$. However, a strong decrease in the amount of $L_{RC}^{30.6PC}$ after five months, and of $L_R^{38.9PC}$ and $\gamma^{40.3}$-subunit after six months of darkness, due to the strong increase in the ratio of the $\gamma^{37.3}$-subunits to 48-53% in the last two months of darkness, indicating the loss of some rods containing PC and PE in $PBS_{up}$.

After one month of re-illumination in $PBS_{up}$, the proportions of all linker polypeptides were fully recovered. Moreover, the amount of the $\gamma^{33.5}$-subunit increased drastically, and a double ratio of 23.9 % was achieved in December, indicating the coupling of further PE. However, in the Antarctic summer months (January-March) the amount of $\gamma^{33.5}$-subunits strongly decreased again.

***Linker polypeptides of the core ($L_{CM}$ and $L_C$)***

Seasonal changes of the large core membrane linkers ($L_{CM}^{75.4}$ and $L_{CM}^{85.0}$), were expressed as % of total PBS protein and are shown in Publ. 5 (Fig. 4 B and E). During darkness, the $L_{CM}^{75.4}$ comprised about 1.5 % of total PBS protein and the $L_{CM}^{85.0}$ about 0.3 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$. After re-illumination from January on, equal amounts of $L_{CM}^{75.4}$ and $L_{CM}^{85.0}$ were found in $PBS_{up}$ and $PBS_{low}$.

The seasonal changes in the small core linker $L_C^{10.0}$ and in the 8.0 kDa polypeptide expressed as % of total PBS protein are shown in Fig. 4 C and F (Publ. 5). $L_C^{10}$ made up about 0.2-0.4 % of total PBS protein, remaining more or less constant during the entire year, both in $PBS_{up}$ and $PBS_{low}$. The 8.0 kDa polypeptide was found in the first part of

the year, between March and September, and made up about 0.1-0.2 % of total PBS protein, both in $PBS_{up}$ and $PBS_{low}$.

*Contaminants*

The seasonal changes of the polypeptides thought to be contaminants (expressed as % of total PBS protein stainable with Coomassie) are shown in Fig. 6 (Publ. 5). The 65.3 kDa and 53.3 kDa polypeptides were present over the entire year. The 53.3 kDa polypeptide increased strongly after re-illumination in $PBS_{low}$ and $PBS_{up}$. The 60.7 kDa, 56.6 kDa and 47.9 kDa polypeptides were found only in the first part of the year, whereas the 57.9 kDa and 50.2 kDa polypeptides appeared only during darkness and after re-illumination. A strong increase in the 57.9 kDa and 52.0 kDA polypeptide was noticed in the last two months of darkness.

# 5. DISCUSSION

## 5.1. Characterisation of phycobilisomes

***Double banded phycobilisomes ($PBS_{up}$ and $PBS_{low}$)***

One interesting result of this work is the finding of two hemiellipsoidal phycobilisome forms of different aggregation states ($PBS_{up}$ and $PBS_{low}$) in *Palmaria decipiens,* and their variation in appearance according to seasonally changing light conditions. Different physiological functions of $PBS_{up}$ and $PBS_{low}$ were found, when their seasonal appearance was related to the phycobiliprotein tissue content and to photosynthetic performance, as discussed in the following chapters.

The isolation of one phycobilisome type from the unicellular microalgae *Porphyridium cruentum* and *Rhodella violacea* using the same protocol excluded the possibility, that the double band is an extraction artefact or the result of partial proteolytic degradation. The appearance of two phycobilisome bands seems not growth temperature specific as two phycobilisome forms were also isolated from the temperate *Palmaria palmata* grown at 10 °C. However, two species of phycobilisomes with different aggregation states and different phycobiliprotein content were also found in *Porphyridium purpureum,* and their variation in response to irradiance and nitrogen availability was demonstrated (Algàrra and Rüdiger 1993). The presence of two phycobilisome types (hemiellipsoidal and hemidiscoidal) has been reported for *Porphyra umbilicalis* collected from the field (Algarra et al. 1990), and for *Porphyra yezoensis* during its life cycle (Shi et al. 1995). In two species of cyanobacteria belonging to the genus *Phormidium* the presence of hemidiscoidal and hemiellipsoidal phycobilisomes, according to the light quality, was observed (Westermann et al. 1993, Westermann and Wehrmeyer 1995). In the above studies, the authors found considerable differences with respect to the phycobiliprotein composition and the structure of the two PBS types. In contrast, in *P. decipiens* two hemiellipsoidal PBS forms were found, which represent two aggregation states probably of different stability. $PBS_{low}$ is a closer packed and $PBS_{up}$ a little more loosely aggregated hemiellipsoidal phycobilisome form. The seasonal acclimation experiments also indicates, that the differentiation between $PBS_{up}$ and $PBS_{low}$ is due to differences in aggregation rather than in phycobilisome size, since no differences in the phycobiliprotein ratios between $PBS_{up}$ and $PBS_{low}$ were found. The higher density of the phycobilisomes is not

due to a higher content of PE, but probably due to interactions with other compounds (Algarra and Rüdiger 1993).

***Phycobiliproteins of $PBS_{up}$ and $PBS_{low}$***

The identification of the phycobiliproteins RIII-phycoerythrin, RI-phycocyanin and allophycocyanin in *P. decipiens* extend the results of Czeczuga (1985) on the same species. The R-phycoerythrin type has three variants (RI-III) and is wide-spread within the Florideophyceae (Honsell et al. 1984, Rennis and Ford 1992). The three R-PE variants show slight spectral differences caused by the various chromophore compositions of their phycobiliproteins (Stadnichuk 1995). Recently a novel R-PE, called R-PE IV, was discovered in the Antarctic red alga *Phyllophora antarctica* MacColl et al. 1996, MacColl et al. 1999).

***The r-phycoerythrin subunit complex***

The trimeric 1-PE subunit ($(\alpha\beta)_3$ or $(\alpha\beta)_3\gamma^{33.5}$)complex was suspected to be r-phycoerythrin, which has been previously described only in few species, like in *Griffithsia pacifica* (Gantt and Lipschultz 1980) and in *Audouinella saviana* (Talarico 1990). It forms aggregates of monomers, dimers, trimers or tetramers and has a low molecular mass of only 55-110 kDa compared to R-phycoerythrin with 265 kDa, which is suspected to form two hexameric aggregates. R-PE and r-PE occurred in a ratio of 4:1 and the quantum yield of r-phycoerythrin is only one-third as large as that of R-phycoerythrin. It is comparable with b-phycoerythrin found in *Porphyridium cruentum* (Gantt and Lipschultz 1974, Glazer and Hixon 1977). The functions of these r-and b-phycoerythrins are still unclear (Talarico 1996, Sidler 1994). It has been suggested that b-PE is somewhat less stable than B-phycoerythrin when released from phycobilisomes (Gantt 1990). It is thought that they mediate a closer packing of biliproteins within the phycobilisome (Gantt and Lipschultz 1974, Gantt and Lipschultz 1980). Dubinsky (1992) further suggested that this 'package effect' may help to dissipate excess energy. Bird et al. (1982) found a nitrogen reserve in r- and b-phycoerythrin. It can also be the precursor used for hexameric functional subunits when a rapid PBS formation is needed (Talarico 1990, 1996). A closer packing of the phycobiliproteins within the $PBS_{low}$ from *P. decipiens* would also explain, why no differences in the ratios of the phycobiliproteins were found, but a smaller size of the negatively stained $PBS_{low}$ was measured.

***Three γ-subunits*** ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$)

Another very interesting result, is the finding of three coloured γ-subunits with different molecular masses ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) both in $PBS_{up}$ and $PBS_{low}$ from *P. decipiens*. The number of γ-subunits varies between one and three within red algae species. Three γ-subunits were also found in R-phycoerythrin from *Callithamnion corymbosum* and *Antithamnion spasum* (Stadnichuk et al. 1993) and in B-phycoerythrin from *Porphyridium cruentum* and *Porphyridium* sp. (Redlinger and Gantt 1981, Swanson and Glazer 1990, Ritz et al. 1998). More commonly, two γ-subunits are present in the R-phycoerythrin from several Florideophyceae: *Aglaothamnion neglectum* (Apt et al. 1993), *Audouinella saviana* (Talarico 1990), *Callithamnion byssoides* and *C. roseum* (Yu et al. 1981), *Gastroclonium coulteri* (Klotz and Glazer 1985), *Gracilaria longa* (D'Agnolo et al. 1994) and *Pterocladiella capillacea* (Talarico et al. 1998). In contrast, only a single γ-subunit was identified in R-phycoerythrin from *Palmaria palmata* (Galland-Irmouli 2000) and *Porphyra umbilicalis* (Algarra et al. 1990).

γ-subunits of red algae carry four or five chromophores (PUB and/or PEB) (Stadnichuk 1995, Stadnichuk et al. 1997), whereas in cyanobacteria only one (PUB) (Ong and Glazer 1991, Wilbanks and Glazer 1993) was found. The difference in the spectral pattern around 544 nm of all three hexameric complexes ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$), indicate the presence of different chromophore ratios of PEB and PUB (PEB:PUB) in the respective γ-subunit ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$), similar as observed before in *Aglaothamnion neglectum* by Apt et al. (1993). These authors isolated two γ-subunits ($\gamma_1$, $\gamma_2$) with distinct molecular masses and different chromophore ratios of PEB:PUB and showed that each subunit complex ($(\alpha\beta)_6\gamma_1$, $(\alpha\beta)_6\gamma_2$) exhibited a different spectral pattern at 550 nm. Accordingly, we assume that in *P. decipiens* the γ-subunit of the 3-PE complex ($\gamma^{33.5}$) has a higher ratio of PEB:PUB, 4-PE ( $\gamma^{40.3}$) an equal ratio of PEB:PUB and 5-PE ($\gamma^{37.3}$) a lower ratio of PEB:PUB. The chromophore composition should be proved by reverse-phase high-pressure liquid chromatography as described by Swanson and Glazer (1990) and Stadnichuk et al. (1993).

Moreover, the three γ-subunits and their associated PE hexamers ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$) were characterised by a minor red shift in their absorption maxima and by a minor red shift in their fluorescence emission maxima, which probably improve the energy transfer downhill the rods. Besides their structural function, linker polypeptides are known to be involved in energy transfer. They modulate the spectral properties of the phycobiliprotein trimers and hexamers by interacting with the chromophores or indirectly by changing the chromophore environment (Glazer 1985, Mimuro et al. 1986a and 1986b, Watson et al. 1986, Glazer and Clark 1986).

However, a second lower fluorescence emission maximum at 595nm of the hexameric 3-PE ($(\alpha\beta_6)^{33.5}$) complex was detected. A fluorescence maximum of PE at 605 was also found in a mutant of *Porphyridium*, leaking of PC (while only a shoulder was seen in the wild type), suggesting the presence of some rods consistent only of PE, which are able to transfer the excitation energy directly to APC.

What might be the advantages of three different γ-subunits ? Our results show, that the three γ-subunits in *P. decipiens* induced a minor red shift in the absorption and fluorescence maxima of their forming subunit complexes ($(\alpha\beta)_6\gamma_1$, $(\alpha\beta)_6\gamma_2$), $(\alpha\beta)_6\gamma_3$), which support the energy transfer downhill the rods. Furthermore, three different PE-complexes ($(\alpha\beta)_6\gamma_1$, $(\alpha\beta)_6\gamma_2$, $(\alpha\beta)_6\gamma_3$) might increase the variability of rod length in *P. decipiens* and may be advantageous for a quick variation in phycobilisome size in order to react to rapid environmental changes. Thus, a loss of the terminal PE hexamers was accompanied by a loss of its associated γ-subunit, observed under high light conditions in *Rhodella violacea* (Bernard et al. 1996). Apt et al. (1993) showed, that the level of transcripts encoding the γ-subunit change in different environmental conditions. However, in the following an alteration of the phycobilisome size by coupling and uncoupling of specific γ-subunits and its associated PE complexes during the seasonal changes was demonstrated. The localisation of the three γ-subunits is shown in models of rods and phycobilisomes from *P. decipiens* (Publ. 3, Fig. 8 and 9).

## 5.2. Acclimation of photosynthesis, pigments and phycobilisome assembly to seasonally changing daylengths

### 5.2.1. Seasonal changes in photosynthesis and pigment tissue contents

The results of this study demonstrate a strong relationship between the seasonal pattern of photosynthetic performance and pigment tissue content. Photosynthetic performance ($ETR_{max}$) increased more or less in parallel with all phycobiliproteins (APC, PC and PE) during the entire Antarctic autumn, winter and spring (from April/Mai to October). For this period of time a positive correlation between total phycobiliprotein tissue content ($PB_{tot}$) and photosynthetic performance and a weaker correlation between Chl *a* and photosynthetic performance was found. The seasonal maximum of photosynthetic performance in September/October is followed by a clear maximum in the Chl *a* and phycobiliprotein contents in October/November. These peaks coincides in time with the

natural sea ice break up in the Antarctic Peninsula region between early September and late November, and when *P. decipiens* has to produce biomass and reserves in a short light window (Wiencke 1990b, Weykam and Wiencke 1996). During summer, photosynthetic performance and all pigment levels decreased to their lowest values. However, Weykam and Wiencke (1996) observed in a similar study with *P. decipiens* an optimum in oxygen production one month later, in October/November, and maximal Chl *a* concentrations in December, with an increase during winter. The total phycobiliprotein content showed no clear seasonal pattern. One reason for differences in comparison with the present study may be the low number of replicates and the difference in age of the plants. However, the present study shows that, the seasonal changes in $ETR_{max}$ are more or less consistent with oxygen flux measurements performed in previous studies (Weykam and Wiencke 1996, Weykam et al. 1997), and seem to be even more precise. It should be mentioned, that ETR not always correlates ETR with oxygen measurements in macroalgae (Hanelt and Nultsch 1995, Beer et al. 2000, Franklin and Badger 2001).

The high $F_v/F_m$ value of *P. decipiens* between May and December confirms that the photosynthetic apparatus remains intact during the entire Antarctic mid autumn, winter and spring. Conversely, the decrease of $F_v/F_m$, $ETR_{max}$ and of all pigments during the entire Antarctic summer suggest a degradation of the photosynthetic apparatus, as the summer daylength seem to lead to an excess of light energy (increase in daily light doses). Since *P. decipiens* is adapted to low light (Wiencke 1990b, Wiencke et al. 1993, Weykam et al. 1996), the degradation of light harvesting antennae might protect the alga from the risk of photodamage caused by the prolonged daylength and is part of its life strategy. A seasonal decrease of phycobiliproteins during summer was also found in *Gracilaria verrucosa* and in *Halopithys incurvus* (Kosovel and Talarico 1979, Talarico and Kosovel 1983). Furthermore, a reduction in phycobiliprotein levels in response to higher irradiances has been noted before in other red algae (Waaland et al. 1974, Beer and Levy 1983, Jahn et al. 1984, Levy and Gantt 1988, Chunningham et al. 1989, Algarra and Niell 1990, Sagert and Schubert 2000, Talarico and Maranzana 2000). A decrease in Chl *a* tissue content and in carotinoids was seen as response to high irradiance (Sagert and Schubert 2000). Chl *a* is found in the reaction centres of PS I and PS II and in a special light harvesting complex of PS I (LHC I) (Wolfe et al. 1994a and 1994b, Tan et al. 1995, Gantt 1996, Marquardt and Rhiel 1997). However, 75-80% of the Chl *a* is associated with PS I and about 18-20% with PS II in *P. cruentum* (Gantt 1996); an amount of even 87-92% of Chl bound to PS I was calculated for *Rhodella violacea* (Marquardt et al. 1999). In *Porphyridum cruentum,* the LHC I binds Chl *a*, zeaxanthin and beta-carotene more than LHC I found in green plants (Tan et al. 1995).

### 5.2.2. Seasonal changes in phycobilisome assembly

***Seasonal changes in the amount of $PBS_{up}$ and $PBS_{low}$***

The total phycobilisome number seems to increase from April/May onwards more or less continuously until October/November, related to the seasonal changes in the APC tissue content. The clear changes in the ratios of APC:Chl *a* and of total phycobiliprotein:Chl *a* tissue content, strongly indicates an alteration in the PBS:PSII:PSI proportion. More often a fixed stoichiometry of PS I:PS II: PBS was observed in red algae (Ohki et al. 1987, Chunningham et al. 1989, Chunningham et al. 1991, Sagert und Schubert 2000).

One interesting result of the present study is, that in *P. decipiens* the amount of $PBS_{up}$ and $PBS_{low}$ individually varies with the seasonally changing daylength. The variation in the appearance of $PBS_{up}$ and $PBS_{low}$ during the Antarctic year seems to be coupled with the seasonal changes of daylength and with the seasonal changes of the phycobiliprotein tissue content. During times of lower phycobiliprotein tissue contents, only one of the two phycobilisome forms was present in high concentrations, while the other one almost disappeared (between Antarctic February and May). During the times of higher phycobiliprotein tissue contents, both PBS forms were present in high amounts, but with changing dominance (Antarctic June-January).

The decreasing daylength in February (Antarctic summer) seems to induce the loss of $PBS_{low}$, resulting in a reduction of APC, PC and PE tissue contents by about 50% to their minimal values. Moreover lowest PBS:PSII:PSI proportions were reached. A marked decrease of phycobiliproteins and the absence of phycobilisomes was also found in *Audouinella* at higher irradiances. In turn the carotinoid content increased (Talarico et al. 1991). An increase in carotinoids (lutein much more than beta-carotene) content under higher irradiances was also seen in *Palmaria palmata* (Sagert and Schubert 2000). An increase in lutein in summer is also possible for *P. decipiens*, since the color in the carotinoid band of the sucrose density gradients from $PBS_{up}$ and $PBS_{low}$ changed.

In April, there appears to be a conversion of $PBS_{up}$ into $PBS_{low}$, since the phycobiliprotein tissue content was still considerably low, and especially the APC tissue content was unchanged, but the amount of $PBS_{up}$ decreased and the amount of $PBS_{low}$ increased. In May, when the phycobiliprotein tissue content started to increase, the amount of $PBS_{low}$ increased further. In June and July, the amount of $PBS_{up}$ clearly increased. Thus, *P. decipiens* seems to acclimate to the shortest daylength by increasing the amount of $PBS_{up}$, resulting in the highest PBS:PSII:PSI proportion of the entire year.

In August and September, the APC and PC tissue contents were unchanged, but in September the amount of $PBS_{up}$ decreased to a moderate level for the rest of the year. The amount of $PBS_{low}$ increased, suggesting a conversion of $PBS_{up}$ to $PBS_{low}$. In October, the APC and PC tissue contents increase to their highest values, and $PBS_{low}$ was still much more dominant than $PBS_{up}$. From December onwards, APC and PC content, and the amount of $PBS_{low}$ decreased in a manner, that $PBS_{up}$ and $PBS_{low}$ appeared in nearly equal amounts in December and January. In February, $PBS_{low}$ was almost negligible.
Two phycobilisome populations with different aggregation states ('soluble' and 'aggregated' PBS) and their variation in appearance according to light irradiance and nitrogen avaiblability were also been shown in *Porphyridium purpureum* (Algarra and Rüdiger). The acclimation to low light occurred by increasing the amount and the size of 'soluble' PBS, with parallel decreasing the amount of 'aggregated' PBS. Acclimation to high light occurred by decreasing the amount and the size of the 'soluble' PBS, with parallel increasing of the amount of 'aggregated' PBS, which also decreased in size. However, at the end of the acclimation processes, 77% of total PE tissue content was associated to the 'soluble' PBS form and only 12% to the 'aggregated' PBS form at low light, and the opposite was achieved at high light. These results show, that a conversion of both phycobilisome populations is possible and would support the theory of a conversion of $PBS_{up}$ into $PBS_{low}$ in *P. decipiens*. Algarra and Rüdiger (1993) interpreted, that only the 'soluble' PBS appears to be able to photoacclimate, but not 'aggregated' PBS. The 'aggregated' PBS was predominant under high light and nitrogen limitation.

Since a strong relationship between the seasonal changes of phycobiliprotein tissue content and of photosynthetic performance was found in *P. decipiens*, the variation in the appearance of $PBS_{up}$ and $PBS_{low}$ during the Antarctic year seems to be also related to the seasonal changes in optimal quantum yield and photosynthetic performance. However, in summer (February) the loss of $PBS_{low}$ was accompanied by a marked fall in the Chl *a* tissue content and a clear reduction in optimal quantum yield and photosynthetic performance. In contrast, the conversion from $PBS_{up}$ to $PBS_{low}$ in April was accompanied by an increase in optimal quantum yield, and the increase in the amount of $PBS_{low}$ in May was accompanied by an increase in photosynthetic performance, while the Chl *a* tissue content remained unchanged until June. Furthermore, the conversion from $PBS_{up}$ to $PBS_{low}$ in September, was accompanied by a sudden increase in photosynthetic performance. We suppose, that $PBS_{low}$ stimulates or enhances the photosynthetic performance. In contrast, $PBS_{up}$ appears to be the basic light harvesting antennae or even

a 'pre-phycobilisome' to build up $PBS_{low}$, when an enhancement in photosynthetic performance is required.

***Seasonal changes in the size of $PBS_{up}$ and $PBS_{low}$***

Both, $PBS_{up}$ and $PBS_{low}$ varied their size during the entire year. The phycobilisome size altered by changing the PE content, whereas the PC content remain constant. The invariance in the PC:APC ratios during the entire year, indicate no alterations in the number of rods. The seasonal changes in the PE:APC and PE:PC ratios indicate alterations of the rod length by coupling or uncoupling of PE. A variation of only PE was also described for other red algae during acclimation to different light conditions (Waaland et al. 1974, Kursar et al 1983, Chunningham et al. 1989, Algarra and Rüdiger 1993). In contrast, *Gracilaria verrucosa* acclimates to seasonal changes by changing the phycobilisome number, but without changing the phycobilisome size (constant ratios of PE:APC, PE:PC and PC:APC) (Kosovel and Talarico 1979). *Aglaothamnion neglectum* is another alga, which acclimates to irradiance only by changing the phycobilisome number and not the size (Apt and Grossman 1993).

Both, $PBS_{up}$ and $PBS_{low}$ changed more or less in the same manner their size during the entire year. From April on and clearly from August on, the phycobilisome size increased more or less continuously during autumn, winter and spring to its 'largest' size in November, both in $PBS_{up}$ and $PBS_{low}$. From December on, when almost the maximal daylength was reached, both $PBS_{up}$ and $PBS_{low}$ clearly started to decrease in size by continuously uncoupling of PE during the hole summer. The prolonged daylength seems to lead to an excess of light energy (increase in daily light doses), and may introduce the reduction in the phycobilisome size. Similarly, a decline in the PE:APC and/or PE:PC ratio was observed during acclimation to high irradiance (Waaland et al. 1974, Levy and Gantt 1988, Chunningham et al. 1989).

Another important point of this study is, that the phycobiliprotein ratios resulted from the phycobiliprotein tissue content and the speculated changes in phycobilisome size (Publication 2) do not correlate with the present results. This supports the strong need to investigate phycobilisomes and not only phycobiliprotein tissue contents of red algae.

***Seasonal changes in the rods ($\gamma$-subunits, $L_R^{38.9PC}$, and $L_{RC}^{30.6PC}$)***

Alteration in the rod length was recognised in the seasonal changes of the percentage ratios (%-ratios) of the linker polypeptides belonging to the rods ($\gamma$-subunits, $L_R^{38.9PC}$ and $L_{RC}^{30.6PC}$). Both in $PBS_{up}$ and $PBS_{low}$, the $L_{RC}^{30.6PC}$ and the $L_R^{38.9PC}$ did not vary with the seasons. This agrees with the observed constant PC:APC ratios during the entire year.

Clear seasonal changes were seen only in the $\gamma^{33.5}$-subunit, whereas the $\gamma^{40.3}$-subunit did not vary, both in $PBS_{up}$ and $PBS_{low}$. The amount of the predominant $\gamma^{37.3}$-subunit also slightly increased, but only between April and June in $PBS_{up}$. Thus, the 'large' phycobilisome size was achieved primarily by doubling the relative proportion of the $\gamma^{33.5}$-subunit, suggesting an elongation of the rods by an association of the $\gamma^{33.5}$-subunit and its associated PE hexamer. From December on the uncoupling of the $\gamma^{33.5}$-subunit started, probably caused by an excess of light energy, like discussed above. A loss of the terminal PE hexamers with its associated γ-subunit was also found under high light (Reuter and Müller 1993, Bernard et al. 1996).

An alteration of the $\gamma^{33.5}$-subunit and its associated PE hexamer also agrees with the finding of the $\gamma^{33.5}$-subunit in the trimeric PE subunit complex, suspected to be r-phycoerythrin. This r-phycoerythrin would be then the intermediary form during coupling/uncoupling of the hexameric form to/from the phycobilisome, like previously demonstrated with free PE (Algarra and Rüdiger 1993).

***The location of the γ-subunits within the rods***

The smallest $\gamma^{33.5}$-subunit and its associated PE hexamer is probably located at the periphery of the rods. A loss of the γ-subunit with the lowest molecular mass, and its location at the distal end of the rods, was also found in other red algae (Bernard et al. 1996, Apt et al. 2001). In contrast, Ritz et al. (1998) observed the loss of the γ-subunit with the highest molecular mass, in a mutant of *Porphyridium*, whereas the wild type showed all three γ-subunits. Furthermore, in this mutant, the rod-core linker (specific to PC) and phycocyanin were absent, and therefore a clear second PE fluorescence emission peak occurred at 605 nm (while only a shoulder was seen in the wild type). The authors suggested, that the remaining two γ-subunits of lower molecular masses and their associated PE hexamers transfer the excitation energy directly to APC. Consequently the wild type must contain rods, which only consist of PE and transfer the energy with a high efficiency directly to APC. This may also be possible for *P. decipiens*, since the $\gamma^{33.5}$-subunit and its associated PE hexamer showed a fluorescence emission maximum at 582 nm and a second lower maximum at 595 nm. Furthermore, a minimal fluorescence at 618 nm and 632 nm was discovered, why it was suspected to be originally only contamination with PC (Lüder et al. 2001b). Thus, *P. decipiens* might modulate the phycobilisome size by alteration the length of rods, which are only or mainly consisting of PE hexamers associated with the $\gamma^{33.5}$-subunit. By this way a more efficient energy transfer, compared to the other hexameric PE subunit complexes is provided.

From its annual variation, the $\gamma^{37.3}$-subunit should be positioned at the intermediary position within the PE part of the rods. However, its associated PE hexamer fluoresces maximally with a slight red shift at 585 nm (Lüder et al. 2001b), suggesting clearly an inner position.

From its annual variation, the $\gamma^{40.3}$-subunit should be positioned at the innermost PE part of the rod, but its associated PE hexamer fluoresces only maximal at 581 nm (Lüder et al. 2001b), suggesting clearly a peripheral position. Since its associated PE hexamer appeared only in a minimal level, a special position is assumed, like close to the thylakoid membrane and at the periphery of the rods, may be attached to FNR (ferredoxin $NADP^+$ oxidoreductase).

Possible rod linker arrangements are summarised in Fig. 7 (Publ. 3) and a model of the largest phycobilisome from *P. decipens* is presented in Fig. 8 (Publ. 3).

***Seasonal changes of the core ($L_{CM}$ and $L_C$)***

A very interesting feature are the seasonal changes of the two large core membrane linker $L_{CM}^{85.0}$ and $L_{CM}^{75.4}$. 'Small' phycobilisomes (in $PBS_{up}$ and $PBS_{low}$) were characterised by a higher abundance of the smaller $L_{CM}^{75.4}$, whereas the 'large' phycobilisomes were characterised by a higher abundance of the larger $L_{CM}^{85.0}$. This suggests an acclimation or regulation phenomena of the phycobilisome core. Two large polypeptides were often described in phycobilisome isolations and thought originally to be only dissociation products (Algarra and Rüdiger 1993). However in cyanobacteria it was demonstrated, that the molecular mass of $L_{CM}$ directly determines the core structure/size: a $L_{CM}^{72}$ formed a two cylinder core, a $L_{CM}^{94}$ and $L_{CM}^{99}$ a three cylinder core and a $L_{CM}^{128}$ resulted in a five cylinder core (Sidler 1994). Furthermore, per phycobilisome core two copies of $L_{CM}$ are present, and reconstitution experiments showed that they also might be of different molecular masses (Esteban 1993, Gottschalk et al.1994).

Two different $L_{CM}$ ($L_{CM}^{120}$ and $L_{CM}^{105}$) were also found in 'small' phycobilisomes achieved under high light and low temperature, while only a single $L_{CM}$ ($L_{CM}^{120}$) occurred in 'large' phycobilisomes achieved under low light and high temperature in a cyanobacterium (Reuter and Nickel-Reuter 1993). Moreover, at high light irradiance or in red light an increase in an approximately 15 kDa smaller $L_{CM}$ was noticed, whereas its decrease at low light irradiance or in green light was observed. These observations are highly reproducible in cyanobacteria and in red algae; therefore an unspecified proteolytic degradation of the $L_{CM}$ is very unlikely and a post-translational modification of $L_{CM}$ has been suggested (Reuter and Müller 1993). Modifications of the core composition seem also to be involved in acclimation processes. This is a new aspect, since over a long

period of research on phycobilisomes, it has been postulated that the core composition is constant at all culture conditions (Reuter and Müller 1993).
The second linker type involved in the assembly of the core, the small core linker $L_C^{10}$, was present more or less in the same abundance over the entire year. A second low molecular mass polypeptide of 8.0 kDa present only in the first part of the year might be a second core linker, like previously discovered in *Rhodella violacea* (Reuter et al. 1990).

***Seasonal changes of contaminants***
The remaining polypeptides were often found in SDS gels of phycobilisomes. The 60.7 kDa and 65.3 kDa polypeptides, which increased clearly from September on, might be proteases responsible for the coupling/uncoupling of PE.

## 5.3. Acclimation of photosynthesis, pigments and phycobilisome assembly during and after six months of darkness - simulating Antarctic winter sea ice cover

### 5.3.1. The influence of darkness on photosynthesis and pigment tissue content

*Acclimation to darkness.* The clear reduction in $ETR_{max}$ and $F_v/F_m$ from the third month of darkness may be interpreted as the start of the degradation of the photosynthetic apparatus. The phycobiliprotein levels started to decrease after four months and the Chl *a* levels after five months of darkness. In contrast, Weykam et al. (1997) observed in a similar long-term culture study with *P. decipiens* a gradual decrease of photosynthetic oxygen production during darkness and found no major changes in Chl *a*, phycobiliproteins were not measured. One reason for differences in comparison with the present study may be the difference in age of the plants. Nevertheless, both studies showed that after six months of darkness *P. decipiens* lost its ability to photosynthesise.
During darkness, an initialy increase over three months of the phycobiliprotein and Chl *a* tissue contents was noticed. A rise in pigments during the first period of darkness has also been observed in other macroalgae (Sheath et al. 1977, Bird et al. 1982) and in microalgae (Peters 1996, Peters and Thomas 1996). Sheath et al. (1977) further demonstrated that PE and Chl *a* can be synthesised in darkness at rates comparable to those in the light. Increases in pigment concentrations during the first period of darkness

seem to be comparable to photoacclimation processes to low light, but a prolongation over three months of darkness is unusual and may be a feature of the life strategy of *P. decipiens*. In the parallel long-term experiment without dark exposure, the seasonal changes in phycobiliprotein and Chl *a* content showed a continuous rise in mid-autumn and winter and were highest in spring; therefore a photoperiodic control of pigment synthesis triggered by daylength was discussed as previously suggested for growth in *P. decipiens* (Wiencke 1990b, Weykam and Wiencke 1996, Wiencke 1996). Thus, the observed rise in pigments during the first three months of darkness also suggests that the pigment synthesis follows a fixed seasonal pattern and a photoperiodic control of pigment synthesis.

The reduction of Chl *a* levels during the last two months of darkness might point to a degradation of PS II and PS I reaction centres or LHC I. The remaining Chl *a* content was still considerably high, when compared to the Chl *a* content measured before the period of darkness. So we assume, that the extremely low $F_v/F_m$ value of 0.1 indicates a damage of the reaction centre from PSII. The biodegradation and de novo synthesis of D1 protein (the half-time of D1 protein turnover can be as short as 30 min, Aro et al. 1993) is probably disturbed may be through a lack of amino acids; but the synthesis of D1 protein itself is light-dependent and it is well known that only limited repair of photodamaged PS II complexes occurs in darkness (Aro et al. 1993, Melis 1991).

We further observed that, *P. decipiens* develops new blades in June during darkness, corresponding to the begin of the seasonal growth period (Wiencke 1990b). Weykam et al. (1997) discovered new blade formation, however, this was found to occur a bit later in early August. The energy source to survive darkness and to accumulate pigments as well as to start blade formation was stored floridean starch, accumulated during the previous summer (Weykam et al. 1997). One reason for the large decrease in phycobiliproteins from August onwards may be that floridean starch had become a limiting factor and that *P. decipiens* requires the remaining energy to support growth at the expense of photosynthesis. This is supported by a significant decrease in floridean starch content observed in August by Weykam et al. (1997). Furthermore, the phycobiliproteins themselves may be used as a nitrogen reserve to ensure growth (Grossman et al. 1994, Bird et al. 1982, Wyman et al. 1985). Another alternative energy source may be the degradation of thylakoids, as observed during dark incubation by Sheath et al. (1977) and Lüning and Schmitz (1988). But the Chl *a* level in *P. decipiens* was still considerably high at the end of the dark period, which would exclude this option.

*Acclimation to re-illumination.* The time course of re-illumination showed that *P. decipiens* started by 24h after re-illumination to accumulate (may be equal to synthesise) Chl *a* and to photosynthesise. The phycobiliprotein accumulation (may be equal to synthesis) started after a lag time of about seven days. *P. decipiens* reached $ETR_{max}$ values, comparable to the values before darkness, seven days after re-illumination and maximal values after 30 days of re-illumination. After 17 days of re-illumination, $ETR_{max}$ reached a value equivalent to the maximum found in the parallel long-term experiment without darkness. However, a similar rapid Chl *a* synthesis was observed in *Delesseria sanguinea* (Lüning and Schmitz 1988) exposed to darkness for nine months. Even here, the phycobiliprotein content remained unchanged during the first week of re-illumination, but in contrast photosynthetic oxygen production was first detected after three days of re-illumination. A similar rapid recovery of photosynthetic performance was found in only three weeks dark-incubated *Porphyra leucosticta* after one day of re-illumination (Sheath et al. 1977).

A strong relation between pigment content and photosynthetic performance was evident by the positive correlation between Chl *a* content and $ETR_{max}$ as well as between Chl *a* content and $F_v/F_m$ during re-illumination of *P. decipiens*. Lüning and Schmitz (1988) also observed a parallel increase of Chl *a* content and photosynthetic oxygen production during the first week of re-illumination of *Delesseria sanguinea.*

In contrast, the relationship between the total phycobiliprotein content ($PB_{tot}$) and $ETR_{max}$ as well as between $PB_{tot}$ and $F_v/F_m$ during re-illumination of *P. decipiens* followed a logarithmic form. This suggests that above a certain level of phycobiliproteins a further increase leads to no further rise in $ETR_{max}$ and $F_v/F_m$. It is logical for $F_v/F_m$ as long as the value can not increase above 0.6-0.7 in red algae (Büchel and Wilhelm 1993). Talarico (1996) also observed in *Audouinella saviana* (Nemaliales, Rhodophyta) that maximal pigment content does not always necessarily mean maximal photosynthetic activity and concluded that not all available PE functions to optimise photosynthesis. This would occur only in presence of well assembled phycobilisomes. Phycobiliproteins are not always organised in well assembled PBS (Lüning and Schmitz 1988, Foltran et al. 1996, Talarico 1996). During an annual cycle in *Halopithys incurvus* well assembled phycobilisomes were found at low phycobiliprotein levels whereas small and incompletely assembled PBS occurred at high phycobiliprotein levels (Talarico and Kosovel 1983). Furthermore, an accumulation of PE in excess of its requirement for this phycobiliprotein as a light-harvesting pigment was demonstrated in *Synechocochus* sp., a PE-containing marine cyanobacterium, in nitrogen-sufficient cultures when compared to nitrogen-limited cultures (Wyman et al. 1985) as well as in *Porphyridium* sp. (Sivan and

Arad 1993) and in *Gracilaria tikvahiae* (Ramus and van der Meer 1983) in their wild types when compared to their PE-mutants; the PE-mutants showed similar or even higher photosynthetic activities when compared with their wild types. A function as a nitrogen reserve or even as photoprotection again excess light of the excess PE was suggested by Wyman et al. (1985). Foltran et al. (1996) discussed a function as storage material readily available for phycobilisome formation when adaptive response to a decrease light intensity is required. We think also that additional PE in *P. decipiens* might be stored as nitrogen reserve and could also be used in winter to survive darkness when stored floridean starch becomes limited. A storage role for phycobiliproteins would also explain why in December a further increase of phycobiliproteins was seen, whereas concurrently $ETR_{max}$ started to decrease. Furthermore in December, the concentration of PE was twice as high than the maximal value measured the a parallel long-term experiment without darkness.

### 5.3.2. The influence of darkness on phycobilisome assembly

***The influence of darkness on the amount of $PBS_{up}$ and $PBS_{low}$***

*Acclimation to darkness.* No alteration in the total phycobilisome amount and in the PBS:PSII:PSI stoichiometry was seen in the first three months of darkness, related from almost constant APC tissue contents and constant APC:Chl *a* ratios. A decrease in both parameters to minimal values occurred in the last three months of darkness and was accompanied by the strong reduction in $F_v/F_m$ and in photosynthetic performance.

One interesting result of the present study is, that $PBS_{low}$ disappeared after two months of darkness. Since no decrease in the total amount of phycobilisomes and in the phycobiliprotein tissue content was seen, a conversion from $PBS_{low}$ into $PBS_{up}$ is possible, like previously discussed for the other direction in the long-term experiment without darkness. This support the theory, that $PBS_{up}$ function as basic light harvesting antennae, probably to build up $PBS_{low}$, when an enhancement in photosynthetic performance is required. However, the remaining $PBS_{up}$ decreased in its amount in the last three months of darkness.

*Acclimation to re-illumination.* After one month of re-exposure to light, the total phycobilisome number was recovered, and was maximal in December, resulting in maximal APC:Chl *a* ratios. The time scale study showed, that $PBS_{low}$ appeared first after 24 days of re-illumination (data not shown). During the summer months the total

phycobilisome number decreased again. $PBS_{low}$ appeared only in small amounts and was almost negligible in February. This agree with the finding in the long-term experiment without darkness, that mainly the amount of $PBS_{low}$ decreased from December onwards during the Antarctic summer months.

***The influence of darkness on the size of $PBS_{up}$ and $PBS_{low}$***

*Acclimation to darkness.* The size of $PBS_{up}$ and $PBS_{low}$ remain unaltered during darkness, related from more or less constant ratios of PE:APC, PC:APC and PE:PC. Only in the last month of darkness, $PBS_{up}$ clearly loosed rods, which are consistent of PC and PE. The strong increase in the PE:PC ratio suggests, that the remaining rods are mainly consistent of PE.

*Acclimation to re-illumination.* After one month of re-illumination, the phycobilisome size of $PBS_{up}$ was recovered totally, and a further coupling of PE occurred. In contrast, $PBS_{low}$ increased clearly in size one month later, mainly by coupling of PE. The PC content was not fully recovered. However, maximal phycobilisome size was achieved between December and February, both in $PBS_{up}$ and $PBS_{low}$. The PE:APC ratios were even somewhat higher than the values measured in the long-term experiment without darkness.

***The influence of darkness on the rods ($\gamma$-subunits, $L_R^{38.9PC}$, and $L_{RC}^{30.6PC}$)***

*Acclimation to darkness.* The alteration in the phycobilisome size was seen more clearly in the seasonal changes of the percentage ratios (%-ratios) of the linker polypeptides belonging to the rods ($\gamma$-subunits, $L_R^{38.9PC}$ and $L_{RC}^{30.6PC}$). In the first four months of darkness in $PBS_{up}$, the proportions of all linkers were more or less the same, indicating no alteration in the phycobilisome size. Even $PBS_{low}$ showed no changes in the proportions of all linkers during the single month of darkness. However, in the last two months of darkness, in $PBS_{up}$ the amount of the rod core linker $L_{RC}^{30.6PC}$ and one month later of the rod linker $L_R^{38.9PC}$ and the $\gamma^{40.3}$-subunit clearly decreased, indicating a loss of rods containing PC and PE. Consequently, the remaining $\gamma^{37.3}$-subunit increased in its relative proportion. In contrast, the $\gamma^{33.5}$-subunit seems to be unaffected during darkness. This agrees with the finding in the long-term experiment without darkness, that the smallest phycobilisome size was characterised by the smallest amount of the $\gamma^{33.5}$-subunit, found in April just before darkness begun. Since no increase in phycobilisome size was recorded during darkness, even no increase in the amount of the $\gamma^{33.5}$-subunit is suspected.

The degradation of phycobilisomes was described in N- and S-deprived cells of cyanobacteria as an ordered process (Yamanaka and Glazer 1980, Collier and Grossman 1992, Grossman 1994). The degradation started at the periphery of the phycobilisomes with the elimination of the terminal hexamer and its associated linker polypeptide, followed by degradation of the next hexamer and its associated linker polypeptide and subsequently, by the loss of some entire rods. This 'trimming' process was generally complete within 24 hours. The degradation of phycobilisome polypeptides resulted in a decrease in the phycobilisome size and a reduction in the ratio of PC:APC. The smaller phycobilisomes still functioned in harvesting light energy. Continued nutrient deprivation resulted in the complete degradation of the remaining phycobilisome structure. Upon adding the limiting nutrient back to the deprived cultures, new phycobilisomes are rapidly synthesised to normal levels before growth resumes. The phycobilisome degradation seems to be catalysed by a protease, that is synthesised de novo in response to N- and S-deprivation. The increased expression of the small gene *nblA*, found in a 2 kbp fragment of genomic DNA, triggers the phycobilisome degradation (Collier and Grossman 1994).

*Acclimation to re-illumination.* After one month of re-exposure to light, in $PBS_{up}$ $L_{RC}^{30.6PC}$, $L_R^{38.9PC}$ and $\gamma^{40.3}$-subunit were recovered and a strong increase in the amount of the $\gamma^{33.5}$-subunit was evident, indicating an increase of the phycobilisome size by coupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer, like previously observed in the long-term experiment without darkness.

After re-illumination, even in $PBS_{low}$ a strong increase in the amount of the $\gamma^{33.5}$-subunit with maximal values in December occurred, but the $L_{RC}^{30.6PC}$ and probably also the $L_R^{38.9PC}$ were not recovered before February. This supports the theory, that the $\gamma^{33.5}$-subunit and its associated PE hexamer might form rods consistent only of PE, which might directly couple to APC as previously discussed in the long-term experiment without darkness.

However, both in $PBS_{up}$ and $PBS_{low}$ an uncoupling of some $\gamma^{33.5}$-subunit and its accociated PE hexamer was noticed in the Antarctic summer months, as previously observed in the long-term experiment without darkness. Nevertheless, the remaining amount of the $\gamma^{33.5}$-subunit was still extremly high and reached values comparable to the maximal values measured in the long-term experiment without darkness. This might explain, why no strong changes in the PE:APC ratios in January and February were seen. However, a clear reduction was seen in March.

***The influence of darkness on the core ($L_{CM}$ and $L_C$)***

Another interesting point is the changes of the two large core membrane linker $L_{CM}^{85.0}$ and $L_{CM}^{75.4}$. Before and during darkness, 'small' phycobilisomes were characterised by a higher abundance of the smaller $L_{CM}^{75.4}$, both in $PBS_{up}$ and $PBS_{low}$. After re-illumination, the large phycobilisomes found in January and February were characterised by a higher amount of the larger $L_{CM}^{85.0}$ and a lower amount of the $L_{CM}^{75.4}$. This agrees with the finding in the long-term experiment without darkness, and supports the theory of an acclimation or regulation phenomena of the phycobilisome core as previously discussed.

The second linker type involved in the assembly of the core, the small core linker $L_C^{10}$, was present more or less in the same abundance over the entire year. Like previously observed in the long-term experiment without darkness, a second low molecular mass polypeptide of 8.0 kDa was present only in the first part of the year, and was suspected to be a second core linker.

***The influence of darkness on contaminants***

As mentioned above, the remaining polypeptides were often found in SDS gels of phycobilisomes. The 57.9 kDa and 52.0 kDa polypeptides, which increased clearly in the last two months of darkness, might be proteases responsible for the degradation of the phycobisomes.

## 5.4. Conclusion and future outlook

The phycobilisomes of *P. decipiens* possess a broad range of variability. Two hemiellipsoidal phycobolisome forms ($PBS_{up}$ and $PBS_{low}$) of different aggregation behaviour may occur. A conversion of $PBS_{up}$ into $PBS_{low}$ and also in the other direction is possible. Different physiological functions of $PBS_{up}$ and $PBS_{low}$ were discussed. $PBS_{up}$ appears to be the basic light harvesting antennae, whereas $PBS_{low}$ seems to enhance the photosynthetic performance of *P. decipiens*. The number and the size of $PBS_{up}$ and $PBS_{low}$ is variable.

Both in $PBS_{up}$ and $PBS_{low}$, three coloured γ-subunits ($\gamma^{33.5}$, $\gamma^{37.3}$, $\gamma^{40.3}$) and three associated PE-subunit complexes ($(\alpha\beta)_6\gamma^{33.5}$, $(\alpha\beta)_6\gamma^{37.3}$, $(\alpha\beta)_6\gamma^{40.3}$) with different absorption characteristics around 544.5 nm and with a red shift in their absorption maxima were identified. Furthermore, a red shift in their fluorescence emission maxima was detected, which probably improves the energy transfer downhill the rod. Moreover,

the $\gamma^{33.5}$-subunit and its associated hexamer shows an enhanced fluorescence at 595 nm, which further provides the energy transfer downhill the rod and makes a coupling of rods only consistent of PE direct to APC possible. The phycobilisome size is modulated primarily by the coupling and uncoupling of the smallest $\gamma^{33.5}$-subunit and its associated PE hexamer.

A model of possible arrangement of rods and of a phycobilisome was proposed. The $\gamma^{33.5}$-subunit is located at the periphery of the rods. The predominated $\gamma^{37.3}$-subunit is located at the innermost PE part of the rod. The largest $\gamma^{40.3}$-subunit is suspected to be at a special peripheral position, close to the thylakoid membrane, may be attached to FNR.

Furthermore, a trimeric PE subunit complex ($(\alpha\beta)_3$ or $(\alpha\beta)_3\gamma^{33.5}$), with a very low fluorescence signal (compared to the others PE complexes) was identified as r-phycoerythrin, which seems to be the intermediary form during coupling/uncoupling of the hexameric form to/from the phycobilisome.

At last, an acclimation of the phycobilisome core is possible, and the PBS:PSII:PSI stoichiometry is variable.

All together allowed *P. decipiens* to react very rapid and successful to environmental changes in light conditions.

*P. decipiens* is excellently adapted to the short period of favourable light conditions in the field. *P. decipiens* maintains an intact photosynthetic apparatus during the entire mid autumn, winter and spring. The alga starts from April (early Antarctic autumn) onwards to increase the number and the size of $PBS_{up}$ and $PBS_{low}$ more or less continuously until November (Antarctic spring). From July (Antarctic winter) onwards, *P. decipiens* starts to increase Chl *a* tissue contents to built up new reaction centres or even to increase the LHCI. Both leads to an parallel increase of the photosynthetic performance. Subsequently, photosynthetic performance and pigment contents are maximal in Antarctic spring, when the water is very clear and the sunlight penetrates deep into the water column, after break up of sea ice and before the water becomes turbid due to plankton blooms and glacial melt water. In this short light window, *P. decipiens* has to produce biomass and reserves (Wiencke 1990b, Weykam and Wiencke 1996). From December on, the number of $PBS_{low}$ and the size of $PBS_{up}$ and $PBS_{low}$ decreases more or less continuously during the summer months. During Antarctic summer, *P. decipiens* reduces its photosynthetic apparatus to a minimum: maximal quantum yield, maximal photosynthetic performance, phycobiliprotein and Chl *a* tissue contents are all minimised. These results underline the life strategy of *P. decipiens* as a season anticipator.

The analysis of the phycobilisome assembly showed, that the presence of $PBS_{low}$ seems to enhance the photosynthetic performance. Thus, *P. decipiens* seems to enhance photosynthetic performance initially in April by conversion of the existing $PBS_{up}$ into $PBS_{low}$ and increasing the amount of $PBS_{low}$. In June, July and August (Antarctic autumn/winter), *P. decipiens* acclimates to the shortest daylength mainly by increasing the amount of $PBS_{up}$, resulting in a maximal PBS:PSII:PSI stoichiometry. In September $PBS_{up}$ is conversed into $PBS_{low}$, which seems to enhance photosynthetic performance to its maximal values in September/October. In December, with the decrease in the amount of $PBS_{low}$ the photosynthetic performance started to decrease. In summary, *P. decipiens* acclimates to the shortest daylength mainly by increasing the amount of $PBS_{up}$, but also by a slight increase in the size of $PBS_{up}$ and $PBS_{low}$. *P. decipiens* acclimates to the longest daylength and in the summer months mainly by decreasing the amount of $PBS_{low}$, but also by decrease in the size of $PBS_{up}$ and $PBS_{low}$. The phycobilisome size was altered mainly by coupling and uncoupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. The $\gamma^{37.3}$-subunit varies also a little during the entire year, whereas the $\gamma^{40.3}$-subunit seems to be unaffected.

The present study also demonstrated, that *P. decipiens* is very well adapted to a prolonged dark period experienced in the field. In the first three months of darkness, the photosynthetic apparatus was more or less unaffected. The total amount of phycobilisomes remain unchanged and no alteration in the phycobilisome size of $PBS_{up}$ and $PBS_{low}$ was seen. After two months of darkness the $PBS_{low}$ disappeared and was probably conversed into $PBS_{up}$. In the last three months of darkness a clear disruption of the photosynthetic apparatus occurred, indicated by a strong reduction in $F_v/F_m$, photosynthetic performance, and phycobiliprotein tissue contents, due to a reduction in the amount of $PBS_{up}$. A degradation of the remaining $PBS_{up}$ started in the last two months of darkness by a loss of hole rods containing PE and PC. Even the Chl *a* tissue content started to decrease in the last two months of darkness, indicating a degradation of some reaction centres and may be also of the LHCI complexes. After six months of darkness *P. decipiens* loses its ability to photosynthesise. Nevertheless, *P. decipiens* starts very soon, already 24h after re-illumination, to accumulate Chl *a* and to photosynthesise, which would assume a quick repair mechanisms. The phycobiliprotein accumulation (may be equal to synthesis) starts with a lag time of about seven days. The recovery of the photosynthetic performance is accelerated by the accumulation of pigments and the build up of new $PBS_{up}$, expressed in the correlation found between pigment contents and $F_v/F_m$ as well as $ETR_{max}$ during re-illumination. Likewise, *P. decipiens* reaches after seven days

of re-illumination $ETR_{max}$ values comparable to the values before darkness and maximal values after 30 days of re-illumination in November, in Antarctic spring under the best light conditions, like previously discussed. However, the $PBS_{low}$ appeared first after 24 days of re-illumination, and in the following only in small amounts. The size of $PBS_{up}$ was fully recovered after one month of re-illumination, whereas the size of $PBS_{low}$ one month later and without fully recovering of the PC content, suggesting the coupling of rods only containing PE. However, maximal size of $PBS_{up}$ and $PBS_{low}$ was found between December and February and occurred by coupling of the $\gamma^{33.5}$-subunit and its associated PE hexamer. In summer, *P. decipiens* reduces its photosynthetic performance and pigment contents, like previously observed under seasonal changing light conditions, probably to avoid photodamage caused by excess light energy.

*P. decipiens* shows a high potential to acclimate to seasonal changing light conditions and to prolonged darkness. The seasonal changes in pigments and photosynthesis seems to follow a fixed seasonal pattern and suggest the hypothesis of a photoperiodic control of pigment synthesis and of photosynthesis as previously suggested for growth in *P. decipiens* (Wiencke 1990b, Weykam and Wiencke 1996, Wiencke 1996).

This study clearly indicates the strong need to investigate phycobilisomes of Antarctic macroalgae or generally of the more advanced red algae belonging to the Florideophyceae. The unexpected finding of two phycobilisome forms, their variation in occurrence and their conversion represent a new acclimation strategy. Furthermore, the finding of a phycoerythrin subunit complex fluorescing with a strong red shift, which might also be coupled directly to APC, is a new aspect. Even the acclimation of the phycobilisome core is a new feature.

Further investigations are necessary. How is the appearance of two phycobilisome forms in *P. decipiens* influenced by irradiance ? Do both phycobilisome forms occur in all tissue developmental stages and in different algal ages ? How are the large core linkers influenced by irradiance ? A screening of phycobilisome isolations of advanced red algae would be a very interesting study.

# 6. REFERENCES


Algarra P, Niell FX (1990) Short-term pigment response of *Corallina elongata* Ellis et Solander to light intensity. Aquat Bot 36:127-138

Algarra P, Rüdiger W (1993) Acclimation processes in the light harvesting complex of the red alga *Porphyridium purpureum* (Bory) Drew et Ross, according to irradiance and nutrient availability. Plant Cell Environ 16:149-159

Anderson LK, Toole CM (1998) A model for early events in the assembly pathway of cyanobacterial phycobilisomes. Mol Microbiol 30:467-474

Apt KE, Collier JL, Grossman AR (1995) Evolution of the phycobiliproteins. J Mol Biol 248:79-96

Apt KE, Grossman AR (1993a) Characterization and triscript analysis of the major phycobiliprotein subunit genes from *Aglaothamnion neglectum* (Rhodophyta). Plant Mol Biol 21:27-38

Apt KE, Grossman AR (1993b) Genes encoding phycobilisome linker polypeptides on the plastid genome of *Aglaothamnion neglectum* (Rhodophyta). Photosynth Res 35:235-245

Apt KE, Grossman AR (1993c) The phycobilisome $\beta^{18}$ subunit gene of allophycocyanin is located on the plastid genome in *Aglaothamnion neglectum* (Rhodophyta) and cotranscribed with an unidentified open reading frame. J Phycol 29:716-718

Apt KE, Hoffman NE, Grossman AR (1993) The $\gamma$ subunit of R-phycoerythrin and its possible mode of transport into the plastid of red algae. J Biol Chem 268:16208-16215

Apt KE, Metzner S, Grossman AR (2001) The $\gamma$ subunits of phycoerythrin from red alga: position in phycobilisomes and sequence characterization. J Phycol 37:64-70

Araoz R, Lebert M, Häder D-P (1998) Electrophoretic applications of phycobiliproteins. Electrophoresis 19:215-219

Aro EM, Virgin I, Andersson B (1993) Photoinhibition of photosystem II. Inactivation, protein damage and turnover. Biochim Biophys Acta 1143:113-134

Bald D, Kruip J, Rögner M (1996) Supramolecular architecture of cyanobacterial thylakoid membranes: How is the phycobilisome connected with the photosystems ? Photosynth Res 49:103-118

Beer S, Larsson C, Poryan O, Axelsson L (2000) Photosynthetic rates in *Ulva* (Chlorophyta) measured by pulse amplitude modulated (PAM) fluorometry. Eur J Phycol 35:69-74

Beer S, Levy I (1983) Effects of photon fluence rate and light spectrum composition on growth, photosynthesis and pigment relations in *Gracilaria* sp.. J Phycol 19:516-522

Bernard C, Etienne A-L, Thomas J-C (1996) Synthesis and binding of phycoerythrin and its associated linkers to the phycobilisome in *Rhodella violacea* (Rhodophyta):compared effects of high light and translation inhibitors. J Phycol 32:265-271

Bernard C, Thomas JC, Mazel D, Mousseau A, Castets AM, Tandeau de Marsac N (1992) Characterization of the genes encoding phycoerythrin in the red alga *Rhodella violacea*: evidence for a splitting of the *rpeB* gene by an intron. Proc Natl Acad Sci USA 89:9564-9568

Bird KT, Habig C, DeBusk T (1982) Nitrogen allocation and storage patterns in *Gracilaria tikvahiae* (Rhodophyta). J Phycol 18:344-348

Bischof K, Hanelt D, Wiencke C (1999) Acclimation of maximal quantum yield of photosynthesis in the brown alga *Alaria esculenta* under high light and UV radiation. Plant Biol 1:435-444

Brejc K, Ficner R, Huber R, Steinbacher S (1995) Isolation, crystallization, crystal structure analysis and refinement of allophycocyanin from the cyanobacterium *Spirulina platensis* at 23 Å resolution. J Mol Biol 249:424-440

Bryant DA (1991) Cyanobacterial phycobilisomes:progress toward complete structural and functional analysis via molecular genetics In: Bogorad L, Vasil IK (eds) The Photosynthetic Apparatus: Molecular Biology and Operation. Academic Press, San Diego, pp 257-300

Bryant DA, Guglielmi G, Tandeau de Marsac N, Castets AM, Cohen-Bazire G (1979) The structure of cyanobacterial phycobilisomes: a model. Arch Microbiol 123:113-127

Büchel C, Wilhelm C (1993) In vivo analysis of slow chlorophyll fluorescence induction kinetics in algae: progress, problems and perspectives. Photochem Photobiol 58:137-148

Clarke A, Holmes LJ, White MG (1988) The annual cycle of temperature, chlorophyll and major nutrients at Signy Island, South Orkney Island, 1969-82. Br Antarct Surv Bull 80:65-86

Clayton MN (1994) Evolution of the Antarctic marine benthic algae. J Phycol 30:897-904

Clayton MN, Wiencke C (1986) Techniques and equipment for culturing Antarctic benthic marine algae, and for preparing specimens for electron microscopy. Ser Cient INACH 34:93-97

Collier JL, Grossman AR (1992) Chlorosis induced by nutrient deprivation in *Synechococcus* sp. Strain PCC 7942: not all bleaching is the same. J Bacteriol 174:4718-4726

Collier JL, Grossman AR (1994) A small polypeptide triggers complete degradation of light-harvesting phycobiliproteins in nutrient-deprived cyanobacteria. EMBO J 13:1039-1047

Cunningham FX Jr, Dennenberg RJ, Jursinic PA, Gantt E (1990) Growth under red light enhances photosystem II relative to photosystem I and phycobilisomes in the red alga *Porphyridium cruentum.* Plant Physiol 93:888-895

Cunningham FX Jr, Dennenberg RJ, Mustardy L, Jursinic PA, Gantt E (1989) Stoichiometry of photosystem I, photosystem II, and phycobilisomes in the red alga *Porphyridium cruentum* as a function of growth irradiance. Plant Physiol 91:1179-1187

Cunningham FX Jr, Mustardy L, Gantt E (1991) Irradiance effects on thylakoid membranes of the red alga *Porphyridium cruentum.* An immunocytochemical study. Plant Cell Physiol 32:419-426

Czeczuga B (1985) Light-harvesting phycobiliprotein pigments of the red alga *Leptosomia simplex* from the Antarctic. Polar Biol 4:179-181

D'Agnolo E, Rizzo R, Paoletti S, Murano E (1994) R-phycoerythrin from the red alga *Gracilaria longa.* Phytochemistry 35:693-696

Daniels, C (1992) Saisonale Untersuchungen zur Photosynteseaktivität und zur Pigment- und Mannitolkonzentration der endemisch antarktischen Makroalgen *Desmarestia anceps* und *Himantothallus grandifolius* (A et ES Gepp) Zinova. Diploma Thesis, University of Bremen, Germany: pp 90

Drew EA, Hastings RM (1992) A year-round ecophysiological study of *Himantothallus grandifolius* (Desmarestiales, Phaeophyta) at Signy Island, Antarctica. Phycologia 31:262-277

Dubinsky Z (1992) The functional and optical absorption cross sections of phytoplankton photosynthesis. In: Falkowski PG, Woodhead AD (eds) Primary Productivity and Biogeochemical Cycles in the Sea. Plenum Press, New York, pp 31-45

Ducret A, Müller SA, Goldie KN, Hefti A, Sidler WA, Zuber H, Engel A (1998) Reconstitution, characterisation and mass analysis of the pentacylindrical allophycocyanin core complex from the cyanobacterium *Anabaena* sp. PCC 7120. J Mol Biol 278:369-388

Ducret A, Sidler W, Frank G, Zuber H (1994) The complete amino acid sequence of R-phycocyanin-I $\alpha$ and $\beta$ subunits from the red alga *Porphyridium cruentum.* Structural and phylogenetic relationships of the phycocyanins within the phycobiliprotein families. Eur J Biochem 221:563-580

Duerring M, Huber R, Bode W, Ruembeli R, Zuber, H (1990) Refined three-dimensional structure of phycoerythrocyanin from the cyanobacterium *Mastigocladus laminosus* at 27 Å. J Mol Biol 211:633-644

Duerring M, Schmidt GB, Huber R (1991) Isolation, crystallization, crystal structure analysis and refinement of constitutive C-phycocyanin from the chromatically adapting cyanobacterium *Fremyelle diplosiphon* at 166 Å resolution. J Mol Biol 217:577-592

Dunton K (1992) Arctic biogeography:the paradox of the marine benthic faune and flora. Trends Ecol Evol 7:183-189

Egelhoff T, Grossman AR (1983) Cytoplasmic and chloroplast synthesis of phycobilisome polypeptides. Proc Natl Acad Sci USA 80:3339-3343

Esteban MA (1993) Sequenzierung, Klonierung und Characterisierung der Gene von Core-Polypeptiden aus dem Cyanobacterium *Mastigocladus laminosus*. PhD thesis No 10040, Eidgenössische Technische Hochschule, Zürich, Switzerland

Ficner R, Huber R (1993) Refined crystal structure of phycoerythrin from *Porphyridium cruentum* at 0.23-nm resolution and localisation of the γ subunit. Eur J Biochem 218:103-106

Ficner R, Lobeck K, Schmidt G, Huber R (1992) Isolation, crystallization, crystal structure analysis and refinement of B-phycoerythrin from the red alga *Porphyridium sordidum* at 22 Å resolution. J Mol Biol 228:935-950

Foltran A, Maranzana G, Rascio N, Scarabel L, Talarico L, Andreoli C (1996) *Iridaea cordata* from Antarctica: an ultrastructural, cytochemical and pigment study. Bot Mar 39:533-541

Franklin LA, Badger MR (2001) A comparison of photosynthetic electron transport rates in macroalgae measured by pulse amplitude modulated chlorophyll fluorometry and mass spectrometry. J Phycol 37:756-767

Galland-Irmouli AV, Pons L, Luçon M, Villaume C, Mrabet NT, Guéant JL, Fleurence J (2000) One step purification of R-phycoerythrin from the red macroalga *Palmaria palmata* using preparative polyacrylamide gel electrophoresis. J Chromatography B 739:117-123

Gantt E (1981) Phycobilisomes. Annu Rev Plant Physiol 32:327-347

Gantt E (1990) Pigmentation and photoacclimation. In: Cole KM, Sheath RG (eds) Biology of the Red Algae. Cambridge University Press, Cambridge, New York, Port Chester, Melbourne, Sydney, pp 203-219

Gantt E (1996) Pigment protein complexes and the concept of the photosynthetic unit: chlorophyll complexes and phycobilisomes. Photosynth Res 48:47-53

Gantt E, Conti SF (1965) The ultrastructure of *Porphyridium cruentum*. J Cell Biol 26:365-381

Gantt E, Conti SF (1966) Granules associated with the chloroplast lamellae of *Porphyridium cruentum*. J Cell Biol 29:423-434

Gantt E, Conti SF (1967) Phycobiliprotein localization in algae. Brookhaven Symp Biol 19:393- 405

Gantt E, Lipschultz CA (1972) Phycobilisomes of *Porphyridium cruentum*. I. Isolation. J Cell Biol 54:313-324

Gantt E, Lipschultz CA (1974) Phycobilisomes of *Porphyridium cruentum*: Pigment analysis. Biochemistry 13:2960-2966

Gantt E, Lipschultz CA (1980) Structure and phycobiliprotein composition of phycobilisomes from *Griffithsia pacifica* (Rhodophyceae). J Phycol 16:394-398

Gantt E, Lipschultz CA, Grabowski J, Zimmerman BK (1979) Phycobilisomes from blue-green and red algae. Isolation and dissociation characteristics. Plant Physiol 63:615-620

Genty B, Briantais JM, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim Biophys Acta 990:87-92

Giddings TH Jr, Wasmann C, Staehelin LA (1983) Structure of the thylakoids and envelope membranes of the cyanelles of *Cyanophora paradoxa*. Plant Physiol 71:409-419

Gindt YM, Zhou J, Bryant DA, Sauer K (1992) Core mutations of Synechococcus sp PCC 7002 phycobilisomes: a spectroscopic study J Photochem Photobiol B:Biol 15:75-89

Glazer AN (1985) Phycobilisomes: Structure and dynamics of energy flow. In: Steinback KE, Bonitz S, Arntzen CJ, Bogorad L (eds) Molecular Biology of the Photosynthetic Apparatus. Cold Spring Harbour Laboratory, New York, pp 231-240

Glazer AN (1988a) Phycobiliproteins. Methods Enzymol 167:291-303

Glazer AN (1988b) Phycobilisomes. Methods Enzymol 167:304-312

Glazer AN (1989) Light guides. J Biol Chem 264:1-4

Glazer AN, Clark JH (1986) Phycobilisomes: Macromolecular structure and energy flow dynamics. Biophys J 49:115-116

Glazer AN, Hixon CS (1975) Characterization of R-phycocyanin. Chromophore content of R-phycocyanin and C-phycoerythrin. J Biol Chem 250:5487-5495

Glazer AN, Hixson CS (1977) Subunit structure and chromophore composition of rhodophytan phycoerythrins *Porphyridium cruentum* B-phycoerythrin and b-phycoerythrin. J Biol Chem 252:32-42

Glazer AN, West JA, Chan C(1982) Phycoerythrin as chemotaxonomic markers in red algae: A survey. Biochem Systematics Ecol 10:203-215

Gómez I, Thomas DN, Wiencke C (1995) Longitudinal profiles of growth, photosynthesis and light independent carbon fixation in the Antarctic brown alga *Ascoseira mirabilis*. Bot Mar 38:157-164

Gómez I, Wiencke C (1997) Seasonal growth and photosynthetic performance of the Antarctic macroalga *Desmarestia menziesii* (Phaeophyceae) cultivated under fluctuating Antarctic daylengths. Bot Acta 110:25-31

Gottschalk L, Lottspeich F, Scheer H (1994) Reconstitution of allophycocyanin from *Mastigocladus laminosus* with isolated linker polypeptides. Photoche Photobiol 58:761-767

Grossman AR, Schaefer MR, Chiang GG, Colier JL (1994) The responses of cyanobacteria to environmental conditions: light and nutrients. In: Bryant DA (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Netherlands, pp 641-675

Grossman AR, Schaefer MR, Chiang GG, Collier JL (1993) Environmental effects on the light-harvesting complex of cyanobacteria. J Bacteriol 175:575-582

Guglielmi G, Cohen-Bazire G, Bryant DA (1981) The structure of *Gloeobacter violaceus* and its phycobilisomes. Arch Microbiol 129:181-189

Hanelt D (1992) Photoinhibition of photosynthesis in marine macrophytes of the South Chinese Sea. Mar Ecol Prog Ser 82:199-206

Hanelt D (1998) Capability of dynamic photoinhibition in Arctic macroalgae is related to their depth distribution. Mar Biol 131:361-369

Hanelt D, Huppertz K, Nultsch W (1992) Photoinhibition of photosynthesis and its recovery in red algae. Bot Acta 105:278-284

Hanelt D, Jaramillo J, Nultsch W, Senger S, Westermeir R (1994) Photoinhibition as a regulative mechanism of photosynthesis in marine algae of Antarctica. Ser Cient INACH 44:67-77

Hanelt D, Nultsch W (1995) Field studies of photoinhibition show non-correlations between oxygen and fluorescence measurements in the Artic red alga *Palmaria palmata*. J Plant Physiol 145:31-38

Heywood RB, Whitaker TM (1984) The Antarktic marine flora. In: Laws (ed) Antarctic Ecology. Academic Press, London, pp 373-419

Holzwarth AR (1991) Structure-function relationships and energy transfer in phycobiliprotein antennae. Physiol Plant 85:518-528

Honsell E, Kosovel V, Talarico L (1984) Phycobiliprotein distribution in Rhodophyta: Studies and interpretations on the basis of their absorption spectra. Bot Mar 27:1-6

Houmard J, Tandeau de Marsac N (1988) Cyanobacterial genetic tools: Current status. Methods Enzymol 167:808-847

Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll *a* and *b* in N,N-dimethylformamide and 80% acetone. Plant Physiol 77:483-485

Jahn W, Steinbiss J, Zetsche K (1984) Light intensity adaptation of the phycobiliprotein content of the red alga *Porphyridium*. Planta 161:536-539

Jones RF, Speer HL, Kury W (1963) Studies on the growth of the red alga *Porphyridium cruentum*. Physiol Plant 16:636-643

Kain, JM (1989) The seasons in the subtidal. Br Phycol J 24:203-215

Katoh T (1988) Phycobilisome stability. Methods Enzymol 167:313-318

Kim B-K, *Fujita Y (1997) Nucleotide sequence analysis of the phycoerythrin encoding genes in Porphyra yezoensis and Porpyra tenera*. Phycol Res 45:217-222

Kirst GO, Wiencke C (1995) Ecophysiology of polar algae. J Phycol 31:181-199

Klöser H, Ferreyra G, Schloss I, Mercuri G, Laturnus F, Curtosi A (1993) Seasonal variation of algal growth conditions in sheltered Antarctic bays: the example of Potter Cove (King George Island, South Shetlands). J mar Systems 4:289-301

Klotz AV, Glazer AN (1985) Characterization of the bilin attachment sites in R-phycoerythrin. J Biol Chem 260:4856-4863

Kosovel V, Talarico L (1979) Seasonal variations of photosynthetic pigments in *Gracilaria verrucosa* (Huds) Papenfuss (Florideophyceae-Gigartinales). Boll Soc Adr Sci 63:5-15

Kursar TA, Alberte RS (1983) Photosynthetic unit organization in a red algae. Plant Physiol 72:409-414

Kursar TA, van der Meer J, Alberte RS (1983) Light-harvesting system of the red alga *Gracilaria tikvahiae*. II. Phycobilisome characteristics of pigment mutants. Plant Physiol 73:361-369

Lamb IM, Zimmermann MH (1977) Benthic marine algae of the Antarctic Peninsula .Antarct Res Ser 23:129-229

Lange W, Wilhelm C, Wehrmeyer W, Mörschel E (1990) The supramolecular structure of photosystem II - Phycobilisome-complexes of *Porphyridium cruentum*. Bot Acta 103:250-257

Levy I, Gantt E (1988) Light acclimation in *Porphyridium purpureum* (Rhodophyta):Growth, photosynthesis, and phycobilisomes. J Phycol 24:452-458

Lichtlé C, Thomas JC (1976) Étude ultrastructurale des thylakoides des algues à phycobiliproteines, comparison des résultats obtenus par fixation classique et cryodécapage. Phycologia 15:393-404

Lüder UH, Knoetzel J, Wiencke C (2001a) Acclimation of photosynthesis and pigments to seasonally changing light conditions in the endemic Antarctic red macroalga *Palmaria decipiens*. Polar Biol 24:598-603

Lüder UH, Knoetzel J, Wiencke C (2001b) Two forms of phycobilisomes in the Antarctic red macroalga *Palmaria decipiens* (Palmariales, Florideophyceae). Physiol Plant 112:572-581

Lüder UH, Knoetzel J, Wiencke C (2002) Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmaria decipiens* (Rhodophyta) – a study to simulate Antarctic winter sea ice cover. J Phycol 38:904-913

Lüning K (1990) Seaweeds. Their environment, biogeography and ecophysiology. Wiley & Sons, Inc, New York, Chichster, Brisbane, Toronto, Singapore, pp 527

Lüning K, Schmitz K (1988) Dark growth of the red alga *Delesseria sanguinea* (Ceramiales): lack of chlorophyll, photosynthetic capability and phycobilisomes. Phycologia 27:72-77

MacColl R, Guard-Friar D (eds) Phycobiliproteine. CRC Press, Boca Raton, Florida, pp 356

MacColl R (1998) Cyanobacterial phycobilisomes. J Struct Biol 124:311-334

MacColl R, Eisele LE, Malak H, Endres RL, Williams EC, Bowser SS (1999) Studies on R-phycoerythrins from two Antarctic marine red algae and a mesophilic red alga. Polar Biol 22:384-388

MacColl R, Eisele LE, Williams EC, Bowser SS (1996) The discovery of a novel R-phycoerythrin from an Antarctic red alga. J Biol Chem 271:17157-17160

Mandori A, Alhadeff M, Glazer AN, Melis A (1984) Photochemical apparatus organization in *Synechococcus* 6301 (Cyanophyceae). Effects of phycobilisome mutation. Arch Microbiol 139:117-123

Mandori A, Alhadeff M, Glazer AN, Melis A (1985) Phycobilisome-photosystem II association in *Synechococcus* 6301 (Cyanophyceae). FEBS Lett 181:79-82

Marquardt J, Rhiel E (1997) The membrane-intrinsic light-harvesting complex of the red alga *Galdieria sulpuraria* (formerly *Cyanidium caldarium*):biochemical and immunochemical characterization. Biochim Biophys Acta 1320:153-164

Marquardt J, Schultze A, Rosenkranz V, Wehrmeyer W (1999) Ultrastructure and photosynthetic apparatus of *Rhodella violacea* (Porphyridiales, Rhodophyta) grown under iron-deficient conditions. Phycologia 38:418-427

Melis A (1991) Dynamics of photosynthetic membrane composition and function. Biochim Biophys Acta 1058:88-106

Miller KA, Pearse JS (1991) Ecological studies of seaweeds in McMurdo Sound, Antarctica. Am Zool 31:35-48

Mimuro M, Flüglistaller P, Rümbeli R, Zuber H (1986a) Functional assignment of chromophores and energy transfer in C-phycocyanin isolated from the thermophilic cyanobacterium *Mastigocladus laminosus*. Biochim Biophys Acta 848:155-166

Mimuro M, Rümbeli R, Flüglistaller P, Zuber H (1986b) The microenvironment around the chromophores and its changes due to the association states in C-phycocyanin isolated from the cyanobacterium *Mastigocladus laminosus*. Biochim Biophys Acta 851:447-456

Molisch H (1894) Das Phycoerythrin, seine Krystallisierbarkeit und chemische Natur. Botanische Zeitung X:177-191

Molisch H (1895) Das Phycocyanin, ein krystallisierbarer Eiweisskörper. Botanische Zeitung VI:131-135

Mörschel E (1982) Accessory polypeptides in phycobilisomes of red algae and cyanobacteria. Planta 154:251-258

Mörschel E (1991) The light-harvesting antennae of cyanobacteria and red algae. Photosynthetica 25:137-144

Mörschel E, Schatz GH (1987) Correlation of photosystem-II complexes with exoplasmic freeze-fracture particles of thylokoids of the cyanobacterium *Synechococcus* sp.. Planta 172:145-154

Mörschel E, Koller KP, Wehrmeyer W, Schneider H (1977) Biliprotein assembly in the disc-shaped phycobilisomes of *Rhodella violacea*. Eur J Cell Biol 16:118-129

Mörschel E, Mühlethaler K (1983) On the linkage of exoplasmatic freeze-fracture particles to phycobilisomes. Planta 158:451-457

Mörschel E, Rhiel E (1987) Phycobilisomes and thylakoids:The light-harvesting system of cyanobacteria and red algae. In: Harris JR, Horne RW (eds) Membranous Structures (= Electron Microscopy of Proteins, Vol 6). Academic Press, London, Orlando, San Diego, New York, Austin, Boston, Sydney, Tokyo, Toronto, pp 210-254

Nermut MV (1973) Methods for negative staining. In: Schimmel G, Vogel W (eds) Methodensammlung der Elektronenmikroskopie. Wissenschaftliche Verlagsgesellschaft, Stuttgart, Part 3.1.2.3

Nies M, Wehrmeyer W (1980) Isolation and biliprotein characterization of phycobilisomes from the thermophilic Cyanobacterium *Mastigocladus laminosus*. Planta 150:330-337

Ohki K, Okabe Y, Murakami A, Fujita Y (1987) A comparative study of quantitative relationship between phycobiliproteins and photosystem II in cyanobacteria and red algae. Plant Cell Physiol 28:1219-1226

Ong LJ, Glazer AN (1991) Phycoerythrins of marine unicellular cyanobacteria. I. Bilin types and locations and energy transfer pathways in *Synechococcus* spp. phycoerythrins. J Biol Chem 266:9515-9527

Peters E (1996) Prolonged darkness and diatom mortality: II. Marine temperate species. J Exp Mar Biol Ecol 207:43-58

Peters E, Thomas DN (1996) Prolonged darkness and diatom mortality. I. Marine Antarctic species. J Exp Mar Biol Ecol 207:25-41

Provasoli L (1966) Media and prospects for the cultivation of marine algae. In: Watanabe, A and Hattori, A (eds) Cultures and Collections of Algae. Proc US - Japan Conf, Hakone, Sept 1966, Jpn Soc Plant Physiol, 63-75

Ramus J, van der Meer JP (1983) A physiological test of the theory of complementary chromatic adaptation. I. Color mutants of a red seaweed. J Phycol 19:86-91

Redecker D, Wehrmeyer W, Reuter W (1993) Core substructure of the hemiellipsoidal phycobilisome from the red alga *Porphyridium cruentum*. Eur J Cell Biol 62:442-450

Redlinger T, Gantt E (1981) Phycobilisome structure of *Porphyridium cruentum*. Plant Physiol 68:1375-1379

Redlinger T, Gantt E (1982) A Mr 85,000 polypeptide in *Porphyridium cruentum* phycobilisomes and thylakoids: Possible function in linkage of phycobilisomes to thylakoids and in energy transfer. Proc Natl Acad Sci USA 79:5542-5546

Reith M, Munholland J (1993) A high-resolution gene map of the chloroplast genome of the red alga *Porphyra purpurea*. Plant Cell 5:465-475

Rennis DS (1991) Differences in antigenic reactivity of six types of phycoerythrin to monoclonal anti-R-phycoerythrin. Phycologia 30:329-338

Rennis DS, Ford TW (1992) A survey of antigenic differences between phycoerythrins of various red algal (Rhodophyta) species. Phycologia 31:192-204

Reuter W, Müller C (1993) Adaptation of the photosynthetic apparatus of cyanobacteria to light and $CO_2$. J Photochem Photobiol B:Biol 21:3-27

Reuter W, Nickel C, Wehrmeyer W (1990) Isolation of allophycocyanin B from *Rhodella violacea* results in a model of the core from hemidiscoidal phycobilisomes of Rhodophyceae. FEBS Lett 273:155-158

Reuter W, Nickel-Reuter C (1993) Molecular assembly of the phycobilisomes from the cyanobacterium *Mastigocladus laminosus*. J Photochem Photobiol B:Biol 18:51-66

Reuter W, Wehrmeyer W (1988) Core substructure in *Mastigocladus laminosus* phycobilisomes. I. Microheterogeneity in two of three allophycocyanin core complexes. Arch Microbiol 150:534-540

Reuter W, Wehrmeyer W (1990) Core substructure in *Mastigocladus laminosus* phycobilisomes. II. The central part of the tricylindrical core - $AP_{CM}$ - contains the "anchor" polypeptide and no allophycocyanin B. Arch Microbiol 153:111-117

Ritz M, Lichtlé C, Spilar A, Joder A, Thomas J-C, Etienne A-L (1998) Characterization of phycocyanin-deficient phycobilisomes from a pigment mutant of *Porphyridium* sp. (Rhodophyta). J Phycol 34:835-843

Roell MK, Morse DE (1993) Organization, expression and nucleotide sequence of the operon encoding R-phycoerythrin α and β subunits from the red alga *Polysiphonia boldii*. Plant Mol Biol 21:47-58

Rosenberg G (1981) Ecological growth strategies in the seaweeds *Gracilaria foliifera* (Rhodophyceae) and *Ulva* sp (Chlorophyceae). Ph D thesis, Yale University, New Haven, Connecticut

Sagert S, Schubert H (2000) Acclimation of *Palmaria palmata* (Rhodophyta) to light intensity: comparison between artificial and natural light fields. J Phycol 36:1119-1128

Schimer T, Huber R, Schneider M, Bode W, Miller M, Hackert ML (1986) Crystal structure analysis and refinement at 25 Å of hexameric C-phycocyanin from the cyanobacterium *Agmenellum quadruplicatum*. The molecular model and its implications for light-harvesting. J Mol Biol 188:651-676

Schirmer T, Bode W, Huber R (1987) Refined three-dimensional structures of two cyanobacterial C-phycocyanins at 21 and 25 Å resolution - a common principle of phycobilin-protein interaction. J Mol Biol 196:677-695

Schirmer T, Bode W, Huber R, Sidler W, Zuber H (1985) X-ray crystallographic structure of the light harvesting biliprotein C-phycocyanin from the thermophilic cyanobacterium *Mastigocladus laminosus* and its resemblance to globin structures. J Mol Biol 184:257-277

Schluchter WM, Bryant DA (1992) Molecular characterization of ferredoxin-NAPD+ oxidoreductase in cyanobacteria: cloning and sequence of the *petH* gene of *Synechococcus* sp PCC 7002 and studies on the gene product. Biochemistry 31:3092-3102

Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a noninstrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze ED, Caldwell MM (eds) Ecophysiology of Phqtosynthesis (Series: Ecological Studies, Volume 100). Springer Verlag, Berlin, Heidelberg, New York, pp 49-70

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

Sheath RG, Hellebust JA, Sawa T (1977) Changes in plastid structure, pigmentation and photosynthesis of the conchocelis stage of *Porphyra leucosticta* (Rhodophyta, Bangiophyceae) in response to low light and darkness. Phycologia 16:265-276

Shi D-J, Peng G-H, Chen JX, Wang H-J, Jin P, Feng LJ, Zhang H, Fei XG, Tseng CK, Tang PS (1995) Possible occurrence and characteristics of two types of phycobilisomes during ontogenesis of red alga *Porphyra yezoensis*. In: Mathis P (ed) Photosynthesis: from Light to Biosphere, Vol I. Kluwer Academic Publishers, Netherlands, pp 383-386

Shivji MS (1991) Organization of the chloroplast genome in the red alga *Porphyra yezoensis*. Curr Genetic 19:49-54

Sidler W A (1994) Phycobilisome and phycobiliprotein structures In: Bryant, DA (ed) The Molecular Biology of Cyanobacteria. Kluwer Academic Publishers, Netherlands, pp 139-216

Sivan A, Arad S (1993) Induction and characterisation of pigment mutants in the red microalga *Porphyridium* sp (Rhodophyceae). Phycologia 32:38-72

Stadnichuk IN (1995) Phycobiliproteins: determination of chromophore composition and content. Phytochemical Analysis 6:281-288

Stadnichuk IN, Karapetyan NV, Kislov LD, Semenenko VE, Veryasov MB (1997) Two γ-polypeptides of B-phycoerythrin from *Porphyridium cruentum*. J Photochem Photobiol B:Biol 39:19-23

Stadnichuk IN, Khokhlachev AV, Tikhonova YV (1993) Polypeptide γ-subunits of R-phycoerythrin. J Photochem Photobiol B:Biol 18:169-175

Swanson RV, Ong LJ, Wilbanks SM, Glazer AN (1991) Phycoerythrins of marine unicellular cyanobacteria. II. Characterization of phycobiliproteins with unusually high phycourobilin content. J Biol Chem 266:9528-9534

Swanson RW, Glazer AN (1990) Separation of phycobiliprotein subunits by reverse-phase high-pressure liquid chromatography. Analyt Bioche 188:295-299

Talarico L (1990) R-phycoerythrin from *Audouinella saviana* (Nemaliales, Rhodophyta). Ultrastructural and biochemical analysis of aggregates and subunits. Phycologia 29:292-302

Talarico L (1996) Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. Sci Mar 60:205-222

Talarico L, Cortese A, Domevscek D (1991) Response of *Audouinella saviana* (Meneghini) Woelkerling (Nemaliales, Rhodophyta) cultures to light. Oebalia 17:603-613

Talarico L, Kosovel V (1983) Ficobilisomi e variazioni delle ficobiliproteine in *Halopithys incurvus* (Huds) Batt (Rhodomelaceae-Rhodophyta) durante il ciclo annuale. Boll Soc Adr Sci 67:69-81

Talarico L, Maranzana G (2000) Light and adaptive responses in red macroalgae: an overview. J Photochem Photobiol B: Biol 56:1-11

Talarico L, Rascio N, Dalla Vecchia F, Maranzana G (1998) Some observations on phycobilisomes of *Pterocladiella capillacea* (Gelidiaceae, Rhodophyta). Plant Biosystems 132:87-96

Tan S, Wolfe GW, Cunningham FX, Gantt E (1995) Decrease of polypeptides in the PS I antenna complex with increasing growth irradiance in the red alga *Porphyridium cruentum*. Photosynth Res 44:1-10

Tandeau de Marsac N (1991) Chromatic adaptation by cyanobacteria. In: Bogorad L, Vasil IK (eds) The Photosynthetic Apparatus Molecular Biology and Operation (= Cell Culture and Somatic Cell Genetics of Plants, Vol 7B). Academic Press, San Diego, New York, Boston, London, Sydney, Tokyo, Toronto, pp 417-446

Thomas J-C, Passaquet C (1999) Characterization of a phycoerythrin without α-subunits from a unicellular red alga. J Biol Chem 274:2472-2482

Valentin K, Maid U, Emich A, Zetsche K (1992) Organization and expression of a phycobiliprotein gene cluster from the unicellular red alga *Cyanidium caldarium*. Plant Mol Biol 20:267-276

van Thor J J, Mullineaux CW, Matthijs HCP, Hellingwerf KJ (1998) Light harvesting and state transitions in cyanobacteria. Bot Acta 111:430-443

Waaland JR, Waaland SD, Bates G (1974) Chloroplast structure and pigment composition in the red alga *Griffithsia pacifica*: regulation by light intensity. J Phycol 10:193-199

Watson BA, Waaland SD, Waaland JR (1986) Phycocyanin from the red alga *Anotrichium tenue*: Modification of properties by a colorless polypeptide (Mr 30 000). Biochemistry 25:4583-4587

Wehrmeyer W (1983) Organisation and composition of cyanobacterial and rhodophycean phycobilisomes In: Papageorgiou GC, Packer L (eds) Photosynthetic prokaryotes. Cell differentiation and function. Elsevier Biomedical, New York, Amsterdam, Oxford, pp 1-23

Wehrmeyer W, Mörschel E, Vogel K (1993) Core substructure in phycobilisomes of red algae. II The central part of the tricylindrical core - $AP_{CM}$ - a constituent of hemidiscoidal phycobilisomes of *Rhodella violacea*. Eur J Cell Biol 60:203-209

Wehrmeyer W, Zimmermann C, Ohki K, Fujita Y (1988) On a new type of cyanobacterial phycobilisome exemplified in *Phormidium persicinum* Gomont. Eur J Cell Biol 46:539-546

Westermann M, Reuter W, Schimek C, Wehrmeyer W (1993) Presence of both hemidiscoidal and hemiellipsoidal phycobilisomes in a *Phormidium* species (Cyanobacteria). Z Naturforsch Teil C: Biochem Biophys Biol Virol 48:28-34

Westermann M, Wehrmeyer W (1995) A new type of complementary chromatic adaptation exemplified by *Phormidium* sp. C86: changes in the number of peripheral rods and in the stoichiometry of core complexes in phycobilisomes. Arch Microbiol 164:132-141

Weykam G, Gómez I, Wiencke C, Iken K, Klöser H (1996) Photosynthetic characteristics and C:N ratios of macroalgae from King George Island (Antarctica). J Exp Mar Biol Ecol 204:1-22

Weykam G, Thomas DN, Wiencke C (1997) Growth and photosynthesis of the Antarctic red algae *Palmaria decipiens* (Palmariales) and *Iridaea cordata* (Gigartinales) during and following extended periods of darkness. Phycologia 36:395-405

Weykam G, Wiencke C (1996) Seasonal photosynthetic performance of the endemic Antarctic alga *Palmaria decipiens* (Reinsch) Ricker. Polar Biol 16:357-361

Wiencke C (1988) Notes on the development of some benthic marine macroalgae of King George Island, Antarctica. Ser Cient INACH 37:23-47

Wiencke C (1990a) Seasonality of brown macroalgae from Antarctica - a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:589-600

Wiencke C (1990b) Seasonality of red and green macroalgae from Antarctica - a long-term culture study under fluctuating Antarctic daylengths. Polar Biol 10:601-607

Wiencke C (1996) Recent advanes in the investigation of Antarctic macroalgae. Polar Biol 16:231-240

Wiencke C, Clayton MN (2002) Antarctic seaweeds. In: Wägele J, Sieg J (eds) Synopses of the Antarctic Benthos. Volume 9. Koeltz Scientific Books, Königstein, 239 pp

Wiencke C, Rahmel J, Karsten U, Weykam G, Kirst GO (1993) Photosynthesis of marine macroalgae from Antarctica:light and temperature requirements. Bot Acta 106:78-87

Wilbanks SM, Glazer AN (1993) Rod structure of a phycoerythrin II-containing phycobilisome. II. Complete sequence and bilin attachment site of a phycoerythrin γ-subunit. J Biol Chem 268:1236-1241

Wilbanks, S M, Glazer AN (1993) Rod structure of a phycoerythrin II - containing phycobilisome: II. Complete sequence and bilin attachment site of a phycoerythrin γ subunit. J Biol Chem 268:1236-1241

Wolfe GR, Cunningham FX Jr, Durnford D, Green BR, Gantt E (1994a) Evidence for a common origin of chloroplasts with light-harvesting complexes of different pigmentation. Nature 367:566-568

Wolfe GW, Cunningham FX Jr, Grabowski B, Gantt E (1994b) Isolation and characterization of photosystems I and II from the red alga *Porphyridium cruentum*. Biochim Biophys Acta 1188:357-366

Wyman M, Gregory RPF, Carr NG (1985) Novel role for phycoerythrin in a marine cyanobacterium *Synechococcus* DC2. Science 230:818-820

Yamanaka G, Glazer AN (1981) Dynamic aspects of phycobilisome structure: Modulation of phycocyanin content of *Synechococcus* phycobilisomes. Arch Microbiol 130:23-30

Yu M-H, Glazer AN, Spencer KG, West JA (1981) Phycoerythrins of the red alga *Callithamnion*. Plant Physiol 68:482-488

Zhao Z, Zhou J, Bryant, DA (1992) Energy transfer processes in phycobilisomes as deduced from analysis of mutants of *Synechococcus* sp PCC 7002. In Murata N (ed) Research in Photosynthesis, Vol I. Kluwer Academic Publishers, Dordrecht, pp 25-32

Zielinski K (1990) Bottom macroalgae of the Admiralty Bay (King George Island, South Shetlands, Antarctica). Pol Polar Res 11:95-131

Zilinskas BA, Glick RE (1981) Noncovalent intermolecular forces in phycobilisomes of *Porphyridium cruentum*. Plant Physiol 68:447-452

Zuber H (1987) The structure of light-harvesting pigment-protein complexes. In: Barber J (ed) The Light Reactions (= Topics in photosynthesis, Vol 8). Elsevier, Amsterdam, pp 157-259

## ACKNOWLEDGEMENTS

I would like very much to thank:

- my supervisors PD Dr. habil. Jürgen Knoetzel from the Institute for Cell Biology, Biochemistry and Biotechnology at the University of Bremen and Prof. Dr. Christian Wiencke, head of the project group 'Solare UV- radiation' from the Alfred Wegener Institute, for their help and encouragement during all the years.

- Prof. Dr. G. O. Kirst, head of the working group 'Marine Botany' at the University of Bremen, for reviewing this work and being mentor since my time as a biology student.

- Prof. Dr. L. H. Grimme, head of the working group 'Biology with the main focus on Biochemistry and Metabolism' at the Institute for Cell Biology, Biochemistry and Biotechnology, University of Bremen, for taking part in the examination of this thesis.

- all working group members at the University of Bremen, especially my colleagues of the 'photosynthetic unit' Björn Bossmann, Frank Klimmek, Erika Lorenz and Doris Meyer for a nice atmosphere in the lab. Furthermore thanks to Ute Hemboldt for all her technical support.

- all the members of the project group 'Solare UV radiation' as well as those of the working group 'Marine Macroalgae' at the Alfred Wegener Institute for being supportive colleagues, especially Christina Langreder for all her technical support.

- Dr. Jürgen Marquardt from the University of Marburg for technical support.

- Dr. Jürgen Marquardt, Prof. Dr. Igor N. Stadnichuk and Prof. Dr. Laura Talarico for helpful comments on my results that encouraged me to believe in the existence of two phycobilisome forms in *Palmaria decipiens*.